STUDY ON BACTERIOLOGICAL PROFILE OF DIABETIC FOOT INFECTION IN A TERTIARY CARE HOSPITAL

Dissertation Submitted to

The Tamil Nadu Dr. M.G.R. Medical University

In partial fulfillment of the requirement

For the award of the degree of

M.D. (MICROBIOLOGY)

BRANCH IV

APRIL 2013

THANJAVUR MEDICAL COLLEGE, THANJAVUR

THE TAMILNADU DR. M. G. R. MEDICALUNIVERSITY

CHENNAI, TAMILNADU

CERTIFICATE

This is to certify that the dissertation entitled **"STUDY ON BACTERIOLOGICAL PROFILE OF DIABETIC FOOT INFECTION IN A TERTIARY CARE HOSPITAL"** submitted to the Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of regulations required for the award of M.D. Degree in Microbiology is a record of original research work done by Dr. P.Shanmuga Priya, at the Department of Microbiology, Thanjavur Medical College, Thanjavur during the period from April 2011 to April 2012 under my guidance and supervision and the conclusions reached in this study are her own.

Dean

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LIST OF ABBREVIATIONS

ATCC	……..	American Type Culture Collection
BAP	……..	Blood agar plate
CFU	……..	Colony Forming Unit
CLSI	…….	Clinical and Laboratory Standards Institute
CONS	…….	Coagulase Negative Staphylococcus
DFU	……..	Diabetic Foot Ulcer
DFI	……..	Diabetic Foot Infection
DM	.....	Diabetes Mellitus
EDTA	.....	Ethylene diamine tetra acetic acid
ESBL	.....	Extended Spectrum β- lactamase
IDSA	.....	Infectious Disease Society of America
GNB	.....	Gram Negative Bacteria
GPC	.....	Gram Positive Cocci
MHA	……	Muller Hinton Agar
MIC	……	Minimum Inhibitory Concentration
MSA	…….	Mannitol Salt agar
MSSA	……	Methicillin Sensitive Staphylococcus aureus
MRSA	……	Methicillin Resistant Staphylococcus aureus
NAP	…….	Nutrient agar plate
PAD	……	Phenyl Alanine Deaminase
QC	……	Quality Control

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INTRODUCTION

Diabetes mellitus is a health problem of first order as evidenced by the high prevalence and numerous consequences.[1] Approximately 8.3% of the world population suffers from the disease with a similar proportion of undiagnosed patients. Further the incidence increases with age reaching to 11% in above- 65age group. It is the fourth common cause of death all over world as a direct cause not taking into account the cardiovascular mortality due to Diabetes[2].

Diabetic foot infections are frequently occurring, complicated and costly problems in the lifetime of a diabetic.[3] It ranks first among the most common diabetes related cause of lower limb amputation making upto 20% of all hospital admissions and prolonged hospital stay. Approximately 20% of the diabetic patients develop foot problems in the course of their lifetime and illness. To add further to the burden about 40% of them come back for readmission.[4]

Diabetic foot ulcers constitute the most common neurotraumatic cause of amputation[5,6] as about 50% of the patients require a minor or major amputation.[7] There is 15 to 40 fold increased risk of requiring amputation than non-diabetics. Diabetic foot infection increases the need for surgical management like amputation at various levels by 50% when compared to uninfected Diabetic foot ulcers[1].

The mortality rate reported in developed countries in diabetic foot infections is one among six of the diagnosed patients within one year of diagnosis.[8] The burden is obviously under-reported in developing countries due to practical, social and economical grounds.

Survival after amputation is significantly worse than the nondiabetic counterpart of the population, 3- year survival being 50% and 5-year survival being 40 %.[9,10] And the prognosis is worse depending on the level of amputation; higher the level worse the prognosis[10].

The major predisposing factor for diabetic foot infections is presence of ulceration which is often a consequence of disease related neuropathy, vascular disease and compromised immunity[11,12].

DFI has become one of the major medical, social and economical problem all over the world due to its implications on health and hence human resource. It becomes essential to have a detailed clinical study pertaining to the local burden and pattern of the disease. Enumeration of data from such studies in our locality will serve as pillars to support the implementation of modern multidisciplinary approach in management of DFI.

AIM AND OBJECTIVES

AIM

Emphasis the importance of integrated approach towards the diabetic foot management through study of microbiological data in tertiary care hospital.

OBJECTIVES

- To identify and isolate the common organisms causing infection in diabetic foot ulcers.
- To study the Antimicrobial sensitivity pattern of the isolates
- To study the prevalence of Multidrug resistant organisms in the isolates.
- To identify the occurrence of Polymicrobial infection in Diabetic foot infection.
- To observe the risk factors for Polymicrobial infection in Diabetic foot infection.
- To suggest an effective, economical Antimicrobial policy for treatment of Diabetic foot infection.

HISTORICAL REVIEW

"***Diabetes is a wonderful affection, not very frequent among men, being a melting down of the flesh and limbs into urine…. The patients never stop making water, but the flow is incessant, as if from the opening of aqueducts. The nature of the disease, then, is chronic, and it takes a long period to form, but the patient is short-lived, if the constitution of the disease be completely established; for the melting is rapid, the death speedy." -- ARETAES.***

It is understood from the history that the term possibly coined by Apollonius from Memphis in 250BC , the ancient Greek word meaning "**flow through**" as the diseased lost more fluid compared to what they could drink. Mellitus is a Latin terminology meaning **sweetened or honey like** was added latter giving the phrase **"diabetes mellitus".**

The Indian physician Sushruta in 400 B.C. described the sweet taste of urine from affected individuals, and for many centuries to come, the sweet taste of urine was the key to diagnosis.

The earliest description of Diabetes mellitus could be seen in the Hindu writings since 1500 BC. It is described as ***"a mysterious disease causing thirst, enormous urine output, and wasting away of the body with flies and ants attracted to the urine of people."***

Ancient Egyptian history informs about the artificial foot made by them to facilitate the amputated people to walk and the process is well documented in their history. [13]

DEFINITION

WHO definition for Diabetic Foot is that " ***The foot of a patient with diabetes that has the potential risk of developing pathological consequences like infection, ulceration/ destruction of deep tissue often associated with neurologic consequences, various degrees of vascular disease and or metabolic complications of diabetes".***

Boulton in 2002 simply defined Diabetic foot as "***Any foot pathology that results directly from diabetes or its long term complications***"[14]

Any inframalleolar infection in a patient with Diabetes Mellitus which presents as Paronychia, Cellulitis, Myositis, Abcess, Necrotizing fasciitis, Septic arthritis, Tendonitis or Osteomyelitis is simply defined as DIABETIC FOOT INFECTION.[15,16]

RISK FACTORS FOR DIABETIC FOOT INFECTIONS [17]

Risk factors for Diabetic foot ulcer are clearly defined in current literature nevertheless the body of evidence are not as great for risk factors of Diabetic foot infection.

Significant independent risk factors for Diabetic foot infection includes

1. Peripheral neuropathy - Motor, Sensory and Autonomic
2. Vascular (arterial) insufficiency
3. Abnormal anatomy and biomechanics
4. Hyperglycemia and other metabolic derangements
5. Impaired neutrophil function

6. Impaired wound healing and excess collagen cross-linking
7. Disease related disabilities
8. Maladaptive behaviour from the patient side
9. Inadequate healthcare provision

PATHOPHYSIOLOGY OF DIABETIC FOOT INFECTIONS [7]

Although DF lesions may seem different, the path leading to a foot ulcer and its complications is very similar, and is determined by various factors. Understanding of the pathophysiology of Diabetic Foot is essential for optimal care, since modifying the factors that influence its development can restore or keep the foot intact, conserving the limb and maintaining a healthy foot so that the patient can lead a completely normal life.

The predisposing factors for diabetic foot infections are namely neuropathy, vasculopathy and immunopathy.

The most common form of neuropathy is metabolic polyneuropathy, a condition characterized by symmetrical, distal, chronic, insidious onset, somatic and autonomic dysfunction. It predominantly affects the lower extremities[14]. It is found in approximately 30% of diabetics[18], and increases in prevalence with increasing duration of disease[19].

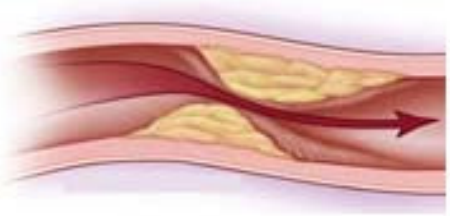
Peripheral neuropathy is an early factor in the pathogenesis & progression of diabetic foot manifestation and is considered the most prominent risk factor in development of foot ulcers in diabetics[20]. It leads to impaired perception and altered response to pain making the patient prone to injuries. Further, by altering gait biomechanics, developing hyperkeratosis (callosities) where plantar pressure is concentrated and ending up in ulcer due to extrinsic factors like improper footwear[21].

Motor component of peripheral neuropathy causes intrinsic muscle weakness and hence imbalance leading to deformities such as clawed digits. The resulting metatarsophalangeal joint instability leads to elevation in plantar pressure. Improper weight distribution marks the beginning of diabetic foot ulcers.

Autonomic dysfunction leads to changes in microcirculation resulting in arteriolar-venous shunting thereby diminishing effective perfusion and elevation of skin temperatures. Concomitant impairment of sebaceous and sweat gland makes the skin dry and thick that cracks and breaks more easily, becoming vulnerable to infection. [22,23]

Sensory neuropathy in the foot and ankle clinic is diagnosed most commonly by testing protective sensation and loss of vibratory sensation[12]. Semmes-Weinstein (10-g) monofilament is used for protective sensation assessment and 128 Hz tuning fork for vibratory sensation.[24]

Diffuse multisegmental macro-angiopathy involving the infrapopliteal vessels, associated with compromised collateral circulation in Diabetic patients is the vascular abnormality in cases of foot pathology. It is similar to atherosclerosis of large vessels in other parts of the body. This presents as arterial insufficiency of the lower extremities compromising normal circulation.[25]



Thickening of the basement membrane in capillary bed referred to as diabetic microangiopathy results in altered nutrient exchange and impaired oxygen transport.[26]

The frequently observed inherent susceptibility to infection in the diabetic patient is attributed to Immunopathy. It has been implicated as the reason for impaired potential to mount a normal inflammatory response to infection and other immunological insults. The molecular pathology behind immunopathy is impaired leukocyte function and altered morphology of macrophages secondary to hyperglycemia.[27]

Leukocyte chemotaxis and phagocytosis is significantly reduced in diabetics and achievement of glycemic control results in improvement of microbiocidal rates.

Impaired chemotaxis of cytokines and excess metalloproteinases create a prolonged inflammatory state that impedes normal wound healing.

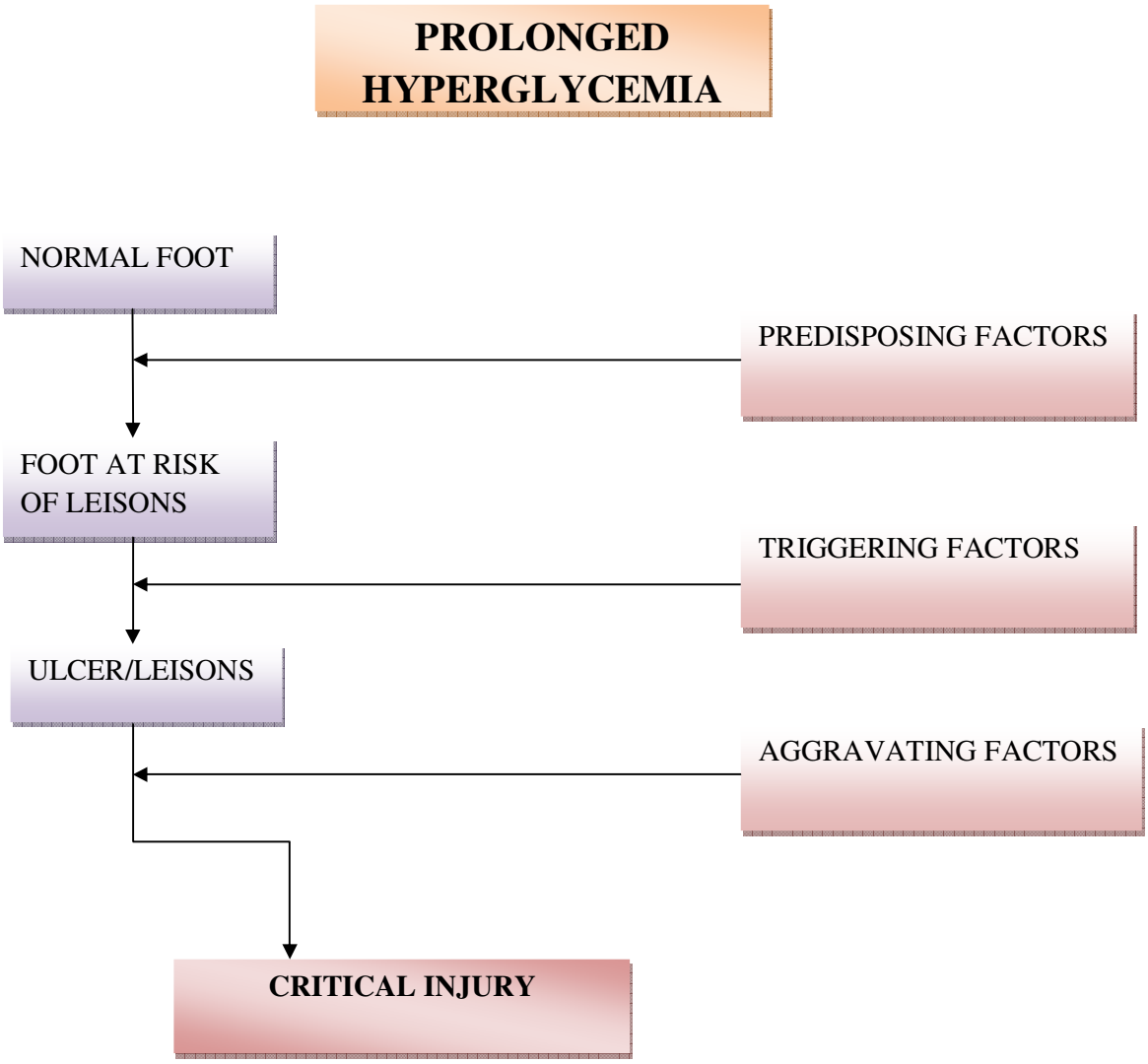
A catabolic state is established by

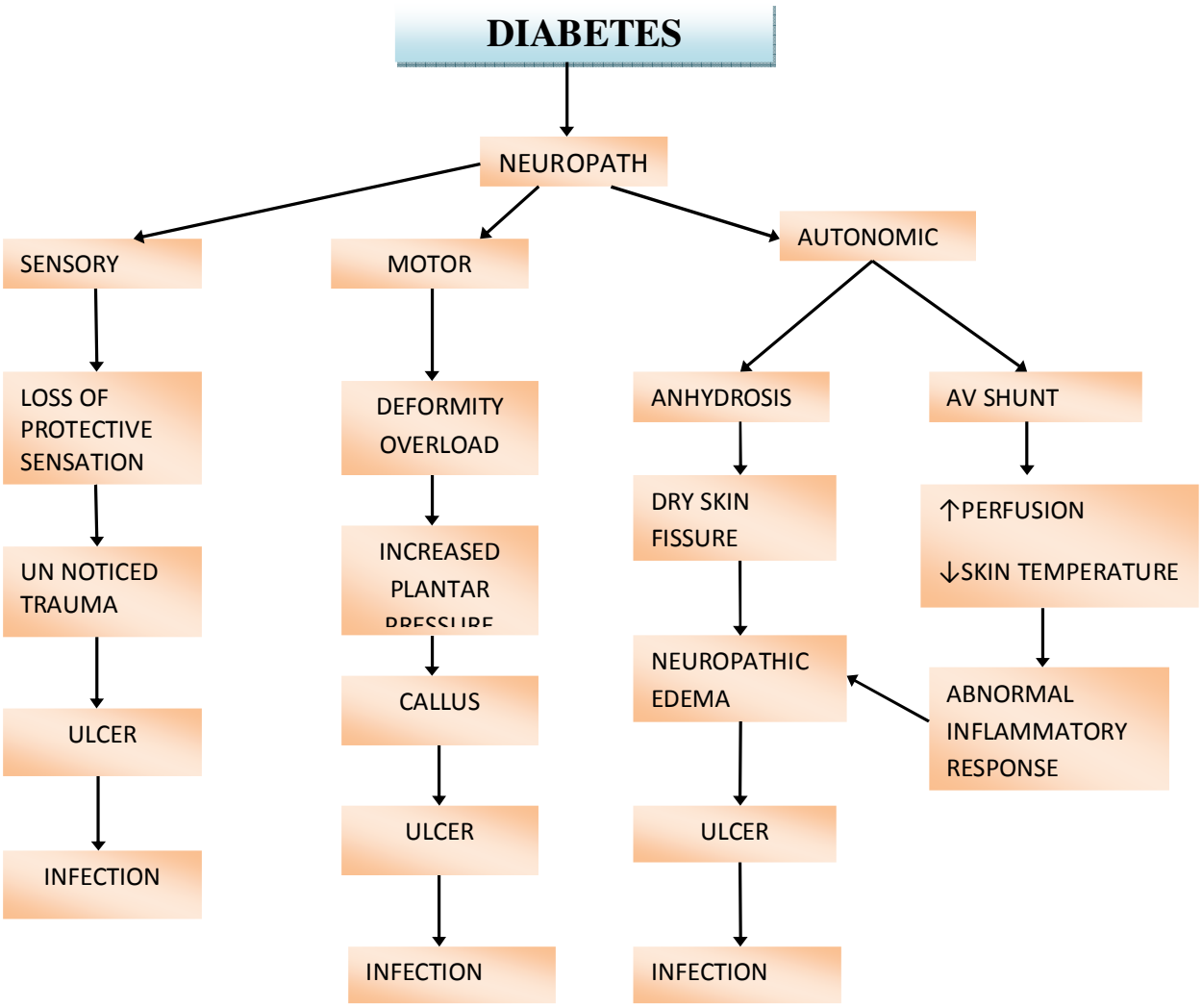
1. Fasting hyperglycemia
2. Presence of an open wound
3. Insulin deprivation and
4. Neoglucogenesis from protein sources.

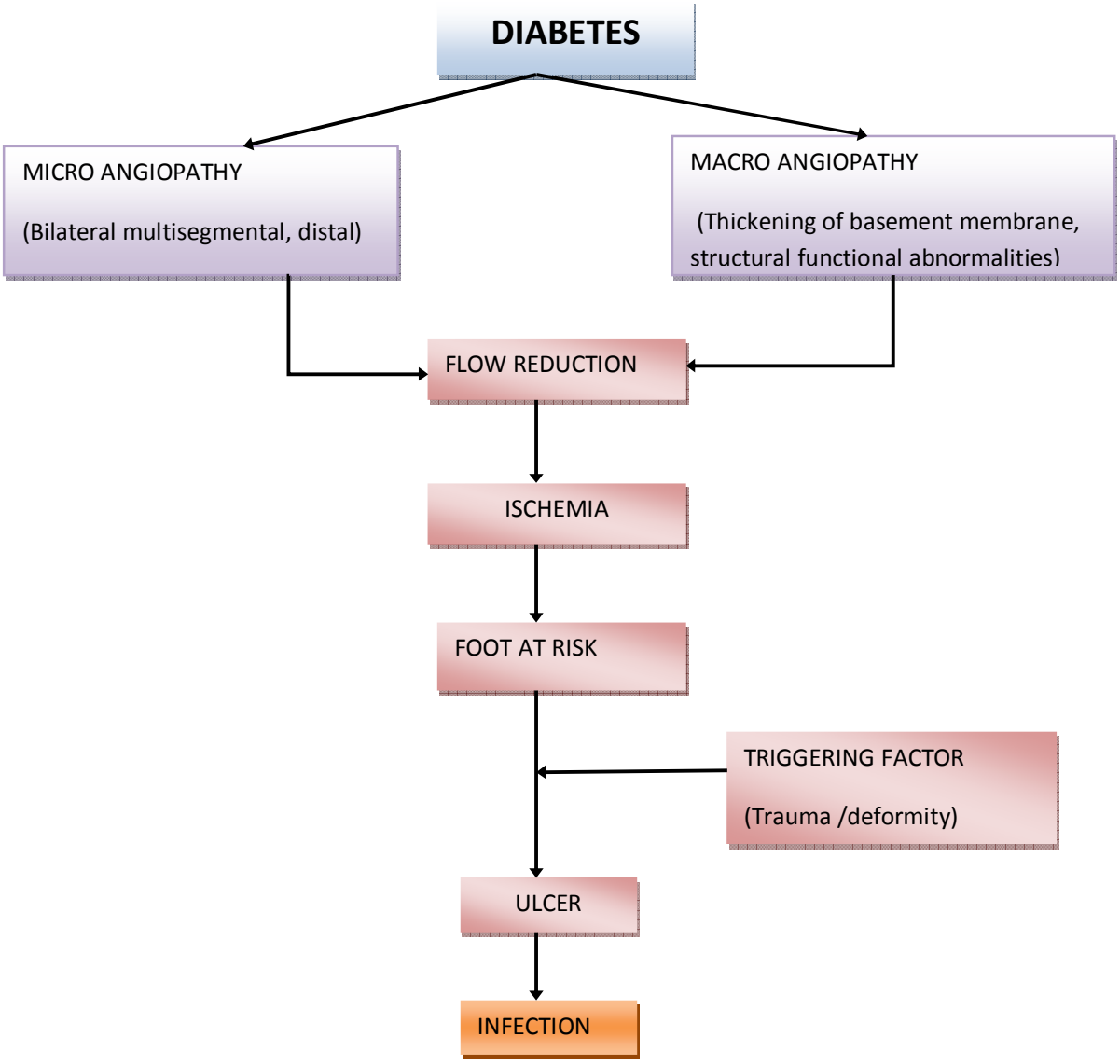
This metabolic alteration impairs protein synthesis, fibroblast proliferation and collagen deposit. Concurrent systemic deficiency of nutrients manifest as delayed wound healing[28].

Field studies show impairment of the immune function with high blood glucose levels (≥150 ml/dl) [29].Diabetics do not fight infection effectively and infection adversely affects glycemic status. This vicious cycle worsens both the underlying disease and the incident complication

FLOW CHART DEPICTING PATHOPHYSIOLOGY OF DFI




DIABETES
NEUROPATH
SENSORY
MOTOR
AUTONOMIC
LOSS OF PROTECTIVE SENSATION
UN NOTICED TRAUMA
ULCER
INFECTION
DEFORMITY OVERLOAD
INCREASED PLANTAR
CALLUS
ULCER
INFECTION
ANHYDROSIS
DRY SKIN FISSURE
NEUROPATHIC EDEMA
ULCER
INFECTION
AV SHUNT
↑PERFUSION
↓SKIN TEMPERATURE
ABNORMAL INFLAMMATORY RESPONSE


DIABETES
MICRO ANGIOPATHY
(Bilateral multisegmental, distal)
MACRO ANGIOPATHY
(Thickening of basement membrane, structural functional abnormalities)
FLOW REDUCTION
ISCHEMIA
FOOT AT RISK
TRIGGERING FACTOR
(Trauma /deformity)
ULCER
INFECTION

EPIDEMIOLOGY

According to an estimate done in 2011 approximately 8.3% of the world population (366 million) suffers from Diabetes making it one of the highly prevalent diseases on earth. A similar proportion of undiagnosed patients have the disease, showing only the tip of the iceberg[30]. Among this around 80% of the population is in developing countries. In addition, there is an increase in incidence of the disease with age, reaching 11% in the persons over 65.[31] The global burden is expected to hike to 9.9% of the adult population i.e. over 552 million in another one or two decade's time. Even in developed countries, it is the 4th cause of death as a direct cause, without taking into account its role in cardiovascular mortality, the leading cause of early death in diabetics.[31,32,33.]

India hosts the majority of diabetic subjects living all over the world. Presently 33 million Indians are victims of diabetes and these scores are likely to reach 57.2 million in another decade. This will account to one sixth of the world diabetes burden. Our country has already become the diabetes capital of the world.

The annual limb loss due to Diabetes is more than 1 million. This means, every 30 second a diabetic person loses a lower limb at some part of the world.

Due to effective treatment protocols diabetic patients having extended life expectancy, report with many problems, including diabetic foot. The main late complications of diabetes like atherosclerosis, neuropathy, retinopathy, etc. are vascular and metabolic in their pathogenesis.

The estimated burden of diabetes mellitus in the United States is more than 25 million and 15-25% of them suffer from foot pathology like diabetic ulcer during their lifetime.[34,35] More than 50% of the ulcers get infected, accounting for high rates of hospitalization, enhanced morbidity and consequent lower extremity amputation.

Diabetic foot infections are the most common diabetes oriented etiology of hospital admissions accounting for 20% of all hospitalizations.[36] Nearly 40% of the discharged patients come for readmission and approximately every one in six patient die within 1 year of development of infectious complication.[37] The decision towards performing amputation is 50% more frequent when compared to diabetic ulcers without infection.[38]

Foot ulcer is one of the most common complications in the lower extremities of diabetics. About 15% of patients experience DFU during the course of disease.[39,40,41] Foot infections affecting the skin, soft tissues and bone with or without systemic impact are the most common reason for hospitalization of diabetics accounting to 25%; with prolonged periods of hospital stay.[40]

Diabetes ranks first as the most common cause of lower extremity amputation in Europe and the U.S.[42] The annual rate of amputations adjusted for age is 82 per 10,000 diabetics. The estimated risk of amputation in diabetics is 15 to 40-fold greater than the nondiabetic counterparts. Men have at least 50% more risk than women .[32,43]

The percentage of diabetics presenting with foot ulcer requiring amputation is 14-20% of total cases. Foot ulcer is the precursor of more than 85% of lower extremity amputations in these patients .[44,45] Post- amputation incidence of a new ulcer, and/or contralateral amputation at 2-5 years is 50%.[40,46]

Survival of diabetic patients undergoing amputations is significantly worse than the rest of the population and even less if they have experienced another prior amputation.[40] Only 50% to 40% of patients survive 3 and 5 years from an amputation, respectively, and prognosis worsens as the level where it is performed increases.[46,47]

EVALUATION OF THE DIABETIC FOOT INFECTIONS[48]

Evaluation of the infection in a diabetic wound is done at three levels,

- General condition of the patient
- Local condition of the limb
- Local condition of the wound per se.

The aim is to assess

- severity of the infection
- cause of the infection
- the pathogenesis of the ulcer
- altered anatomy and biomechanics
- vascular disease
- systemic manifestation of infection

Diagnosis of diabetic foot infection begins with clinical suspicion following a comprehensive elicitation of history and thorough physical examination, supported by complete laboratory evaluation, microbiological assessment and diagnostic imaging procedures.

Diagnosis, correlation and management of diabetic foot infection can be challenging and should utilize the expertise of a multidisciplinary team comprising of surgeons, microbiologists, Diabetology physicians and paramedics.

Initial evaluation of the patient:

1. Basic metabolic panel
2. Complete Hemogram
3. Urine routine examination
4. Blood culture
5. HbA1C
6. C-reactive protein
7. Serum albumin

Evaluation of the Limb:

1. Biomechanics → Deformities (Charcot arthropathy, Claw/hammer toes) ,callosities

Clinical examination & Roentgenogram (≥2 images)

2. Vascular status

Arterial part → Ischemia/ necrosis/ gangrene

Foot pulses, ABI, TcpO2, duplex USG &angiogram

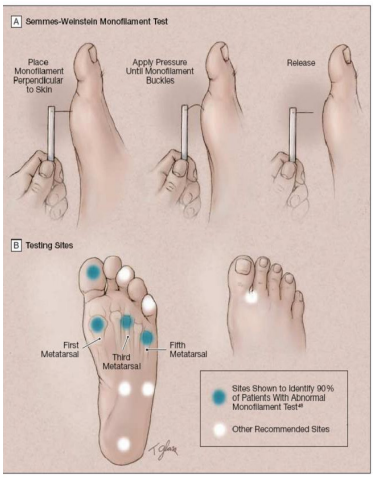
Venous → Edema/ stasis/ thrombosis

Skin & soft-tissue examination and duplex USG

3. Neuropathy ⟶ Loss of protective sensation

↑

Light touch/ monofilament pressure/ vibration perception



EVALUATION OF THE WOUND:

In spite of the fact that there are several classifications being proposed and implemented for the assessment of Diabetic foot ulcers / infections none is universally accepted. The classification schemes incorporate important parameters such as

1. Depth and size of wound
2. Ischemia
3. Peripheral neuropathy
4. Extent of infection.

The following systems of classification are in vogue with their own advantages and limitations.

1. Wagner's classification
2. UT classification
3. The IDSA classification scheme

TABLE 1: Wagner Classification System [49]

GRADE	DESCRIPTION OF ULCER
0	Preulcerative area without open lesion
1	Superficial ulcer (Skin thick)
2	Deep ulcer involving tendon/capsule/bone
3	Stage 2 +Abcess,Osteomyelitis,joint sepsis
4	Gangrene of the wound
5	Gangrene involving entire foot

TABLE 2: IDSA Classification of Severity of Diabetic Foot Infection[50]

IWGDF PEDIS Grade	IDSA Severity of Infection	Clinical Signs of Infection
GRADE 1	NO INFECTION	No inflammatory signs and effusion
GRADE 2	MILD INFECTION	No systemic signs of infection. Evidence of purulence / 2 or more signs of inflammation
GRADE 3	MODERATE INFECTION	No systemic signs of infection. Cellulitis >2cm. Deep tissue infection (crosses subcutaneous cellular tissue, no abscess, lymphangitis, arthritis, osteomyelitis, myositis or critical ischemia)
GRADE 4	SEVERE INFECTION	Any infection associated with systemic toxicity (fever,chills,vomiting,confusion,metabolic instability,shock)

TABLE 3: University of Texas Health Science Center San Antonio classification system [51]

	0	1	2	3
A	No open lesion	Superficial wound	Tendon/capsule	Bone/Joint
B	With infection	With infection	With infection	With infection
C	Ischemic	Ischemic	Ischemic	Ischemic
D	Infection /Ischemia	Infection / Ischemia	Infection / Ischemia	Infection / Ischemia

One of the most widely used Wagner's classification does not take in to account the importance of ischemia and infection as independent risk factors in the classification Grades.

The IDSA classification system consists of four level of infection that can be correlated to clinical findings. Even though it is widely accepted classification system it does not provide the complete description of local wound environment. (15)

A recent classification of Diabetic foot ulcer called The University of Texas Health Science Centre San Antonio (UT) incorporates a matrix structure of four grades of wound depth with subgroups to denote the presence of infection, ischemia or both.

This limitation of IDSA classification system can be surpassed by integration of same with the UT classification for the better understanding of level of ulceration and the existence of ischemia.

Absence of fever does not rule out infection in diabetic patients that may manifest as systemic signs like decreased blood pressure, increased pulse rate, and or severe hyperglycemia. To make the diagnosis even more difficult, more than fifty percent of limp threatening infections do not manifest these systemic signs or symptoms. [52]

The importance of Local examination cannot be underestimated as the tunneling ulcers denote rapidly spreading deep seated infections that do not respect the anatomical planes. [53,54]

Pain and palpation of an otherwise insensate diabetic foot heralds more deep seated infections. As the violation of anatomical barriers is the routine occurrence in diabetic foot ulceration, assessment of dept of ulcer is crucial.

In ulcers showing positive probe to bone examination, the positive predictive value for osteomyelitis is around 95 %.[55,56]

Studies undertaken by abbot et al, Pecoraro et al and the American diabetes association consensus development of on diabetic foot wound have shown a lower positive predictive value of the test. But they came out with 91 percent negative predictive value.

Considering all these study outcome a negative probe to bone test does not rule out a positive correlation with underlying osteomyelitis. [57]

Microbiological Evaluation

A diabetic foot ulcer is considered to be infected when there is purulent discharge, or in the absence of it, the presence of erythema/ heat/ pain/ induration/ tenderness.

DFI almost invariably occurs in patients who suffer an ulcer in their life time[59]. A major cause of infections, often severe, of the lower limb is over infection by gram-positive cocci from skin fissures in the interdigital spaces[17].The diagnosis of the cause of the ulcer is essential in order to plan treatment and enhance healing of the ulcer.

The simple presence of bacteria or any other pathogen is called **contamination**. However the ulcerative bed, rich in protein and other nutritive substances, constitutes a good broth for the microorganisms to reproduce in, leading to the phenomenon of **colonization**.

The following step after colonization is **infection**. It is the tissue invasion of the microorganisms that triggers an inflammatory response with the appearance of the classical local signs and purulent secretion with or without systemic manifestations[60].

The bacterial burden appears to be involved in the transition from colonization to infection. There may be a critical point (10^5 cfu/g of tissue), influenced by the type of microorganism and immune status of the host at which the change from colonization to infection will take place. This is the so-called **critical colonization**[61].

The clinical significance of bacterial colonization lies in the circumstances of chronic nonhealing ulcers despite the absence of overt infection.[62] This is clinically identified as the presence of friable granulation tissue and a serous type secretion.[63] The performance of quantitative cultures may be indicated, with the purpose of detecting that critical colonization which would explain the inadequate course.[64]

This novel microbiological concept of critical colonization has changed the prescription of antimicrobial treatment of chronic ulcers with delayed healing not

explainable by other causes, provided that the quantitative cultures are significant, even at cost of overtreating some patients.

One has to justify the clinical significance of the bacteria isolated as it is clear for highly virulent microorganisms, such as Streptococcus pyogenes, but not for most other recovered species that are usually opportunistic or commensal pathogens.[65]

To address this issue, various solutions like quantitative and semi quantitative methods have been proposed with their own practical, clinical, social and economic limitations.[66,67,68.] It is necessary to have microbiological criteria to assess the qualitative results received by the microbiologists.

The microbial isolates of Diabetic foot infection depend on the type of infection and specific patient situations like antibiotic therapy, previous manipulation or hospitalization.[69,70]

The aerobic gram positive bacteria especially staphylococcus aureus and beta-hemolytic streptococci are the commonest organisms isolated from the previously untreated diabetic foot infection[71]

Patient who underwent treatment with antimicrobials and or with deep seated ulcers are infected by polymicrobial pathogens. [71]

Anaerobic organisms are isolated from patients with mixed infections associated with gangrene[72.]

Isolation of MRSA is more frequent in patients who underwent treatment in hospitals and or who were treated with broad spectrum antimicrobials. Recently the incidence of MRSA is found to be increasing even in the absence of above said conditions due to the increased prevalence of community acquired MRSA infection.[73,74]

MRSA is anticipated in:

1) h/o earlier colonization or previous infection MRSA

2) prevalence of MRSA infection at the locality over10%.

3) if ≥2 of the following conditions are present,

a) h/o hospitalization in the past year or patient is from a healthcare center with endemic MRSA disease

b) treatment with a fluoroquinolone in the previous 6 months

c) > 65 years of age

d) h/o dialysis program for nephropathy.[75]

ESBL- producing E. coli is looked for in

a.> 65 years,

b. female gender

c. hospitalization in the previous year,

d. recurrent urinary tract infection,

e. prior use of fluoroquinolones.

f. Diabetes per se.[76]

Enterococcus spp, CNS, P. aeruginosa, are expected in cases of chronic humid and macerated ulcers receiving multiple treatments.[77]

Common expected pathogens such as S aureus, À-hemolytic streptococci, enterobacteria or anaerobes should be given importance at the point of isolation. Other isolates are considered when they are found in a pure culture or on repeated isolation. No species should be disregarded given the polymicrobial nature of the biofilm of chronic diabetic foot ulcers that warrants attention and repeat cultures in case of an unfavorable course.[78.79,80]

TABLE 4: Infectious Profile of DFI

INFECTION	MICROORGANISMS
Cellulitis	Staphylococcus aureus
Erysipelas	Streptococci
Ulcer untreated with antibiotics	Staphylococcus aureus, Streptococci
Ulcer treated with antibiotic or on long term therapy	MRSA, MSSA, CONS, Streptococci, Enterococci, Enterobacteriaceae, Pseudomonas aeruginosa ,Other nonfermentors, Corynebacterium spp.Candida spp.
Necrotizing fasciitis of myonecrosis(generally polymicrobial)	Anaerobic gram positive cocci Enterobacteriaceae,Non fermenting gram negative bacilli, anaerobes

ANTIBIOTICS in DIABETIC FOOT INFECTION

It has continued to be a controversial issue relating to the institution of empirical antibiotic therapy for Diabetic foot infections. Even positive evidence from microbiology department does not always justify the extensive use of antibiotics. [81]

Clinical criteria suggesting local or systemic infection are taken as supporting factor for such decision. Situations like Osteomyelitis are exceptions when the laboratory data is given weightage.[82].

The Factors to be taken into consideration while planning for an antibiotic regimen are,

1. Efficiecy of the vascular system for effective bioavailability of the drug

2. Immunological Competency of the patient

3. Renal function [83]

Vascular insufficiency and immunological incompetency warrant prolonged periods of administration of bactericidal antibiotics. Anticipation of Renal failure in chronic diabetics leads to avoidance of nephrotoxic drugs, such as aminoglycosides, Vancomycin and amphotericin B [84,85]

The constitution of empirical antimicrobial battery of drugs is based upon the severity of infection, the duration of lesion, previous treatment with antibiotics and local sensitivity pattern.

Gram positive cocci being the predominant pathogens in any circumstance as supported by various global and national studies should always be covered.

RECOMMENDED PROTOCOL

For mild and mild to moderate infections stratified according to the classification systems discussed earlier oral amoxicillin Clavulanic acid is recommended as first line drugs for a period of 7 – 14 days. [69,75]

When MRSA / CONS is suspected the above supplemented with cotrimoxazole or Linezolid.

In beta lactum allergic patients the alternatives are Levofloxacin / Moxifloxacin/ Clindamycin/ cotrimoxazole.

Moderate-severe infections require intravenous administration and hence hospitalization. Antibiotics effective to combat

Staphylococcus spp and Streptococcus spp

Enterobacteriaceae

Streptococcus spp,

Peptostreptococcus spp and Bacteroides spp like

Ertapenem,a third-generation cephalosporin71 plus metronidazole

or amoxicillin-clavulanate may be used140. piperacillintazobactam

can be supplemented if there is suspicion about the involvement of Pseudomonas spp.

In severe infections with systemic impact and lifethreatening

all possibilities should be covered with betalactams with antipseudomonal activity (carbapenem or piperacillin-tazobactam) combined also with daptomycin, linezolid

or vancomycin if there is a risk of MRSA.

Monotherapy with beta-lactams at high doses, namely, a carbapenem, piperacillin-tazobactam or fourth-generation cephalosporin's165 or quinolones (particularly in patients allergic to penicillin) is as effective as combined treatment with aminoglycosides and safer, according to data from nonrandomized clinical series

Failure of a correct antibiotic treatment may be due to the development of resistance, overinfection or extension to bone.We should remember that hospitalized patients and those previously treated with broad-spectrum antibiotics over a long period usually have resistant bacteria.

TABLE 5: Treatment of DFI

Infection	First choice	Alternative
Mild Mild – moderate	Oral amoxicillin – clavulanic acid	Oral levofloxacin or moxifloxacin Oral clindamycin Oral cotrimoxazole Oral linezolid
Moderate severe	IV ertapenem ± IV daptomycin or IV linezolid or IV glycopeptite	IV amoxicillin – clavulanic acid IV 3rd generation cephalosporin+IV metronidazole Or IV fluoroquinolone[2] + IV metronidazole Or IV piperacilln –tazobactam[3] Or IV imepenem or IV meropenem[3] ± IV daptomycin or IV linezolid or IV glycopeptite[1]
Severe	IV imepenem or IV meropenem Or IV piperacilln –tazobactam IV daptomycin or IV linezolid or IV glycopeptite[1]	IV tigecycline + IV fluoroquinolone[2] or IV amikacin

[1] Suspected MRSA [2] Ciprofloxacin or levofloxacin [3] Suspected P.aeruginosa

MATERIALS AND METHODS

PLACE OF STUDY : Thanjavur Medical College Hospital, Thanjavur.

STUDY PERIOD : One year between April 2011 and April 2012

COLLABORATING DEPARTMENTS: Medicine, Surgery, and Diabetology.

DESIGN OF STUDY : Observational study

ETHICAL CLEARANCE : Prior approval obtained from Ethical Committee

INFORMED CONSENT : Obtained from each patient

INCLUSION CRITERIA : Patients with H/O Type II Diabetes mellitus

1. attending Diabetic clinic for DFI/DFU
2. admitted in Sugical wards for DFI/DFU
3. attending surgery OP for DFI/DFU

The patients of all age groups belonging to both the sex with DFU/DFI with or without systemic signs and symptoms of infection were considered and included in the study.

EXCLUSION CRITERIA:

Those with Type I Diabetes mellitus and associated co-morbid conditions, immuno compromised patients, HIV Positive patients were excluded.

SPECIMEN COLLECTION AND TRANSPORT

Specimens for microbiological assessment (frank pus, purulent discharge, serosanguinous discharge) were obtained at the time of admission & at the time of visit to OPD, after thorough vigorous saline wash followed by wound debridement of superficial slough and exudates. Specimens were collected by scraping the ulcer base or the deeper portion of the wound edge with sterile curette into a wide- mouhed sterile container or

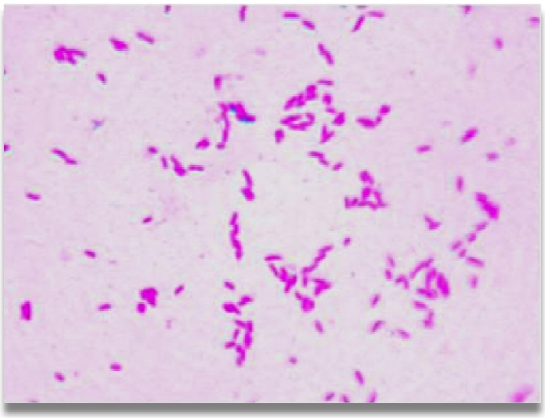
scavenged using sterile swabs and transported to the microbiology lab without undue delay.[85,86]

SPECIMEN PROCESSING

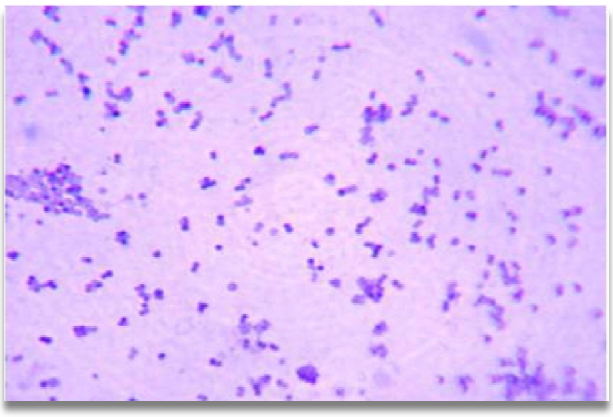
MICROSCOPY

Direct smears are made from the specimens; gram staining was done and examined under oil immersion for the presence of pus cells, epithelial cells, bacteria and fungi and to assess quality of the sample.

GNB IN DIRECT SMEAR



GPC IN DIRECT SMEAR



CULTURE MEDIA USED

1. MacConkey agar
2. Blood agar
3. Nutrient agar

CULTURE METHOD:

Mother inoculum was made with the specimen loaded swab or by loading sterile inoculation loop with the curetted material and Streak culture was done using flame sterilized Nichrome loop. Plates were incubated overnight at 35 - 37°C in ambient air. Culture plates were examined the next day for growth and observations were recorded. The

isolated colonies were identified by adopting the procedures of Gram staining, motility and routine biochemical reactions.

BIOCHEMICAL REACTIONS:

The following tests are routinely done on the isolates.

1. Catalase Test
2. Oxidase Test
3. IMViC Test
4. Urease Test
5. TSI
6. LAO decarboxylation
7. OF Test
8. Sugar Fermentation Reactions
9. Bile esculin hydrolysis
10. Coagulase Test

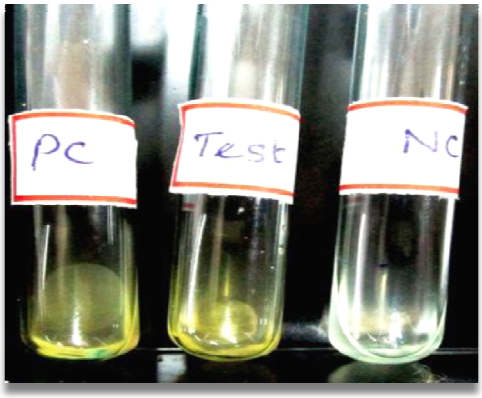
IDENTIFICATION OF STAPHYLOCOCCUS AUREUS

Golden yellow pigmentation

Yellow colonies on MSA

Staphyococcal isolates were identified by the specific colony characters like golden yellow opaque colonies on NAP, β hemolysis on BAP and Coagulase test.

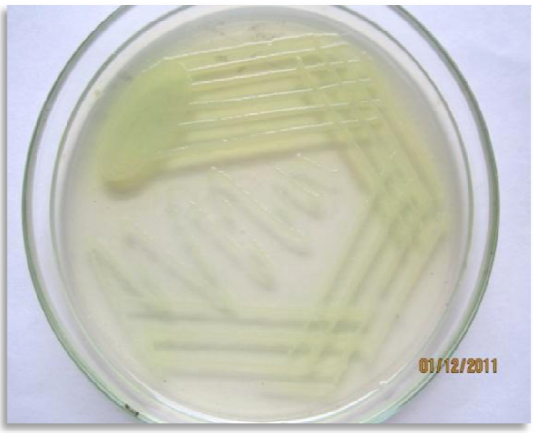
COAGULASE TEST



β hemolysis in blood agar

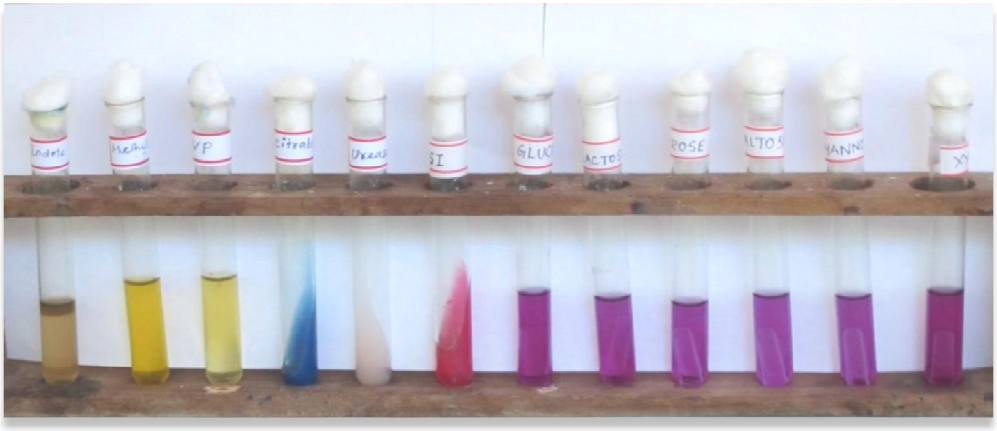
DENTIFICATION OF PSEUDOMONAS AERUGINOSA

Green pigmentation on nutrient agar

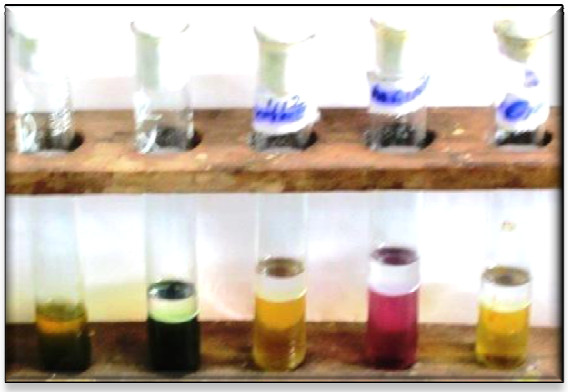


NLF colonies on MacConkey Agar

BIOCHEMICALS FOR P.AERUGINOSA



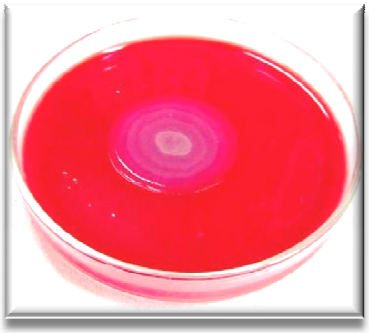
OF & LAO TEST



OXIDASE TEST

DENTIFICATION OF PROTEUS SPP.

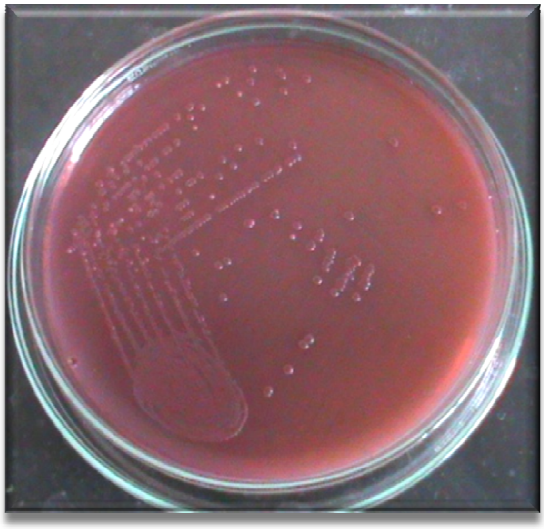
SWARMING ON BLOOD AGAR



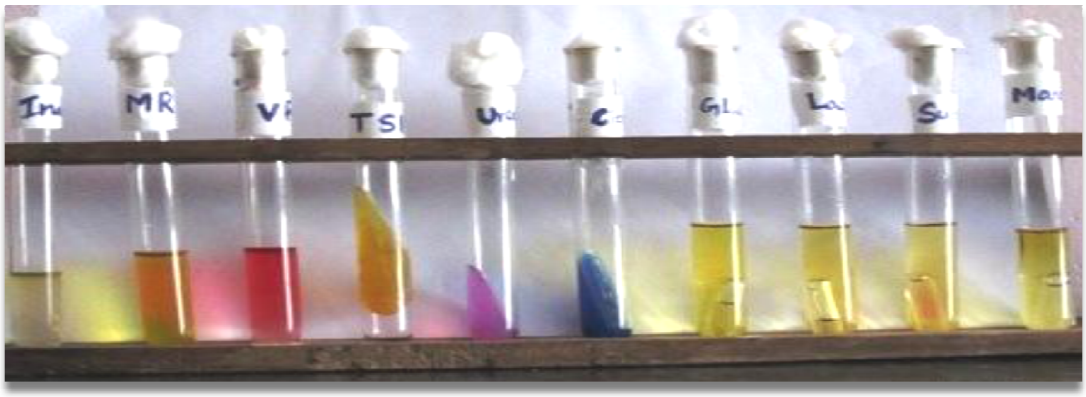
BIO CHEMICAL REACTIONS

DENTIFICATION OF KLEBSIELLA SPP.

KLEBSIELLA SPP.ON MACCONKEY AGAR



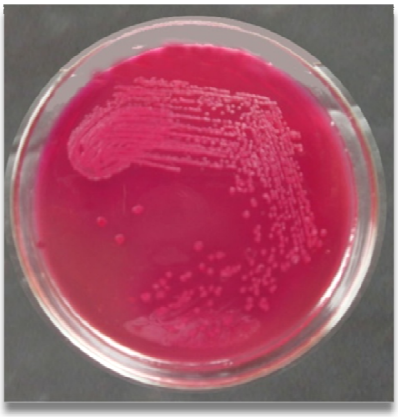
BIO CHEMICAL REACTIONS



ENTEROBACTER SPP. ON MACCONKEY AGAR

BIO CHEMICAL REACTIONS

ESCHERICHIA COLI ON MAC



BIO CHEMICAL REACTIONS

ANTIMICROBIAL SENSITIVITY TESTING[87]

The antimicrobial sensitivity pattern for all the isolates were done in Muller Hinton Agar by modified Kirby – Bauer disc diffusion method as per CLSI guidelines using antibiotic discs (Himedia, Mumbai).

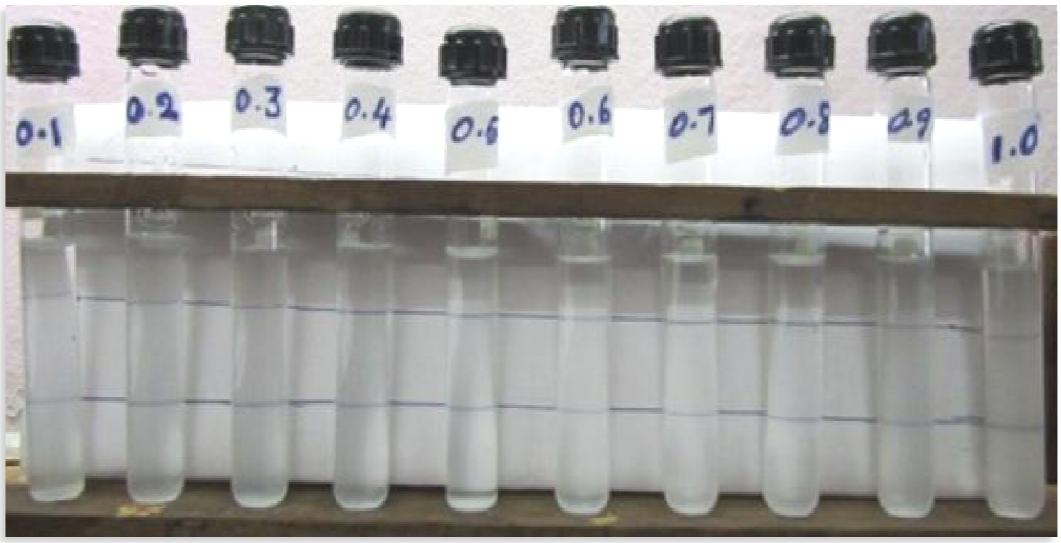
STORAGE OF ANTIMICROBIAL DISCS

All the antimicrobial discs are stored in refrigerator at 4 - 8° C except the β-lactum antibiotics that were stored in the freezer compartment until the day of use.

Discs are brought to room temperature before application. They are replaced in air-tight containers after use.

MCFARLAND TURBIDITY STANDARD [88]

0.5 McFarland turbidity standard is prepared by adding 99.5ml of 1% sulphuric acid and 0.5 ml of 1.175 % barium chloride. Standards ranging from 0.1 to 1.0 are prepared and dispensed in screw-capped test tubes comparable to those used for inoculum preparation, which are sealed tightly and stored in the dark at room temperature. The bacterial suspension that matches with 0.5 McFarland standard provides an inoculum containing approximately 1.5 X 10^{8} CFU/ml.



PREPARATION OF INOCULUM

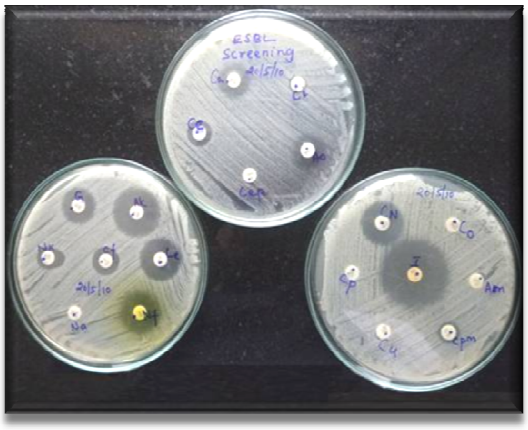
In order to prepare the inoculum**,** about 3-5 representative colonies were picked up and inoculated in 4-5 ml of peptone water and incubated at 37℃ for 2 – 6 hrs to attain turbidity equivalent to 0.5 McFarland's standard which corresponds to 150 million organisms/ml. Turbidity match is done against contrasting black and white background.

INOCULATION OF MHA PLATES

A sterile swab is dipped in the inoculum within 15 minutes of adjusting the turbidity of the inoculum and pressed firmly against the sidewall of the test tube to drain the excess broth. Muller Hinton agar plate is inoculated by streaking the swab over the entire sterile agar surface by streaking two more times and rotating the plates at an angle of approximately 60°c to ensure an even distribution of inoculum. The rim of the agar is swabbed by a circular motion. The closed plate is left for 3-5 minutes to allow any excess surface moisture to be absorbed before applying antimicrobial discs.

APPLICATION OF ANTIMICROBIAL DISCS

The battery of drugs to be applied is determined and the following antimicrobial



discs - Gentamicin, Amikacin, Amoxycillin, Ampicillin, Amoxyclav, Oxacillin, Erythromycin, Cotrimaxazole, Doxycycline, Ciprofloxacin, Ofloxacin, Cephelexin, Cefixime, Ceftazidime, Ceftriaxone, Cefotaxime, Cefipime, Ceftazidime-Clavulanicacid, Aztreonam, Imipenam, and are tested for all the isolates.

Along with the above drugs Azithromycin and Vancomycin are tested for gram positive cocci. Piperacillin-Tazobactum was used only for *E. coli, Klebsiella and Pseudomonas.* The discs are placed on agar plates using thumb forceps and pressed down gently to ensure complete contact with the agar surface. Discs are applied in such a manner that a minimum of 25 mm distance is ensured from centre to centre of the discs. The plates are then incubated at 37° C for 16 – 18 hrs in ambient air.

Control strains are also inoculated following the same procedure.

INTERPRETATION

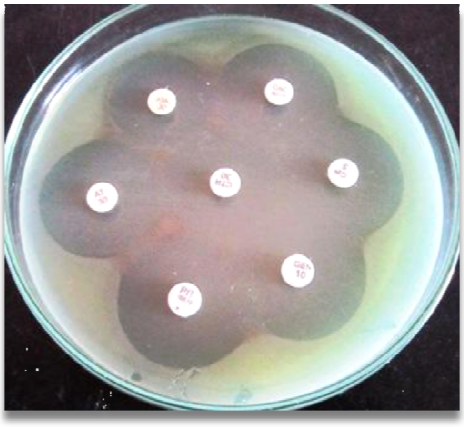
After the stipulated period of incubation, the plates are examined under good light. Satisfactory streaking is confirmed by semiconfluent lawn of growth and uniform circular zones of inhibition.

The zones of complete inhibition from the centre of the discs are measured. The zones are measured to the nearest millimeter using zone scale (Himedia). The Petri plate is held a few inches above a black, non reflecting background illuminated with reflected

light. Zone of inhibition is the margin showing no obvious visible growth detected with naked eyes and interpreted by referring to the CLSI standard guidelines updated from time to time. The organism is reported as sensitive or resistant to the drugs that are tested. An intermediate zone of inhibition is also reported but the clinical application of the data is doubtful.

Control plates were also read using the same procedure and reliability of the test is ensured.

CONTROL STRAINS USED WITH EACH BATCH



ATCC PSEUDOMONOS

Escherichia coli ATCC 25

Pseudomonas aeruginosa ATCC 27853

Staphylococcus aureus ATCC 25923

SCREENING FOR MRSA[89]

The screening for MRSA is done by Oxacillin screen agar method recommended by The National Committee for Clinical Laboratory Standards (NCCLS) containing 6 µg/ml of oxacillin or by disc diffusion on Müeller-Hinton agar supplemented with 4%NaCl .

Spot inoculum over 1-1.5 inch area or routine lawn culture method can be applied. In both methods, any growth after complete 24 hours

of incubation at 35°C in ambient air denotes oxacillin resistance, provided controls are satisfactory.

SCREENING FOR ESBL PRODUCTION[87]

Modified Kirby Bauer disc diffusion method

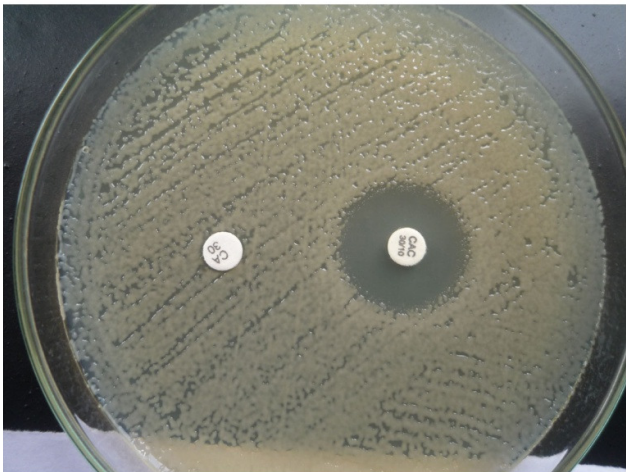
Isolates showing inhibition zones ≤22 mm for Ceftazidime, ≤ 27 mm for Cefotaxime, ≤25 mm for Ceftriaxone, ≤22 mm for Cefpodoxime and ≤27 mm for Aztreonam were identified as potential ESBL producers and they were tested further.

Double disc synergy test[87]

To demonstrate a synergistic action between a 3rd generation Cephalosporin and Clavulanic acid, isolates were grown and adjusted to 0.5 McFarland's standard and lawn culture of it was made on MHA plate.

Discs of 3rd generation Cephalosporin, Cefotaxime (30µg) and Ceftazidime (30µg) were placed 20 mm apart from an amoxicillin (20µg) and Clavulanic acid (10µg) combined disc from centre to centre and incubated at 37°C for 16 – 18 hrs. If inhibition zone around the 3rd generation Cephalosporins showed a clear extension towards Amoxycillin-Clavulanic acid disc then the organisms were said to be ESBL producing.

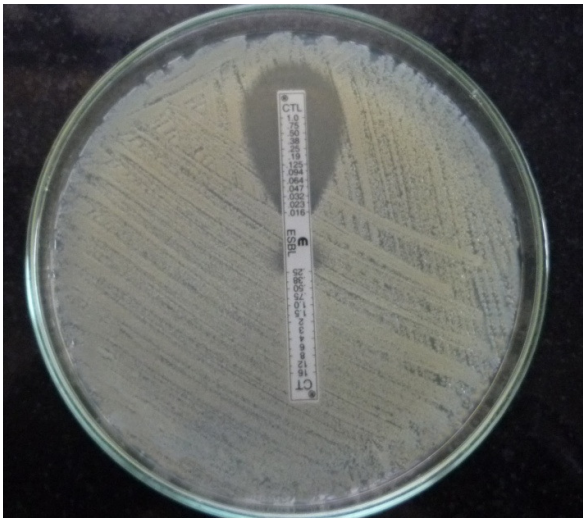
Phenotypic confirmation test



Inhibitor potentiation disc diffusion test (NCCLS confirmatory test)[53]

ESBL production was confirmed by Ceftazidime (30 µg) and Ceftazidime plus Clavulanic acid (30 /10 µg) placed on inoculated MHA plates and incubated. Organism was considered as ESBL producer if there was ≥ 5mm increase in diameter of Ceftazidime/ Clavulanate disc than that of Ceftazidime disc alone.

E-test for ESBL [90,91]



Combination of disc diffusion and Minimum Inhibitory Concentration (MIC) were studied using the E-test strips. The E-test strip contains Ceftazidime gradient at one end and Ceftazidime plus Clavulanate gradient on the opposite end. MHA were inoculated as for disc diffusion and the strips were placed on the inoculated lawn and incubated. MIC was the point of intersection of the inhibition ellipse with the E-test strip edge. Ratio of ceftazidime MIC and Ceftazidime Clavulanic acid MIC ≥ 8 indicated the presence of ESBL.

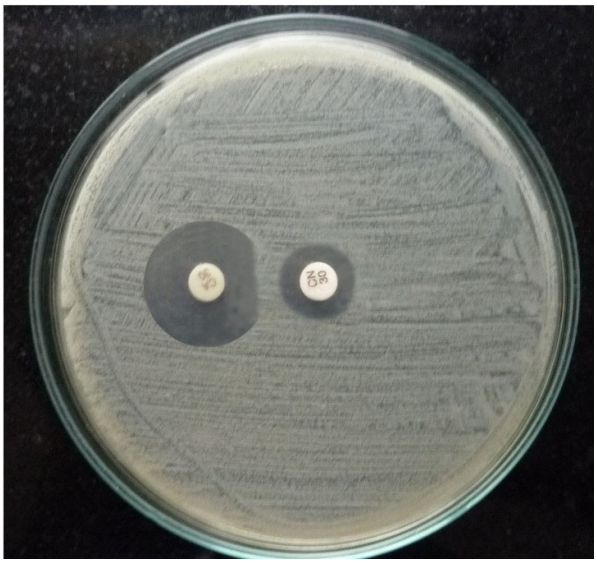
Quality Control (QC) used for ESBL production:

E. coli A7CC 25922 - Negative control

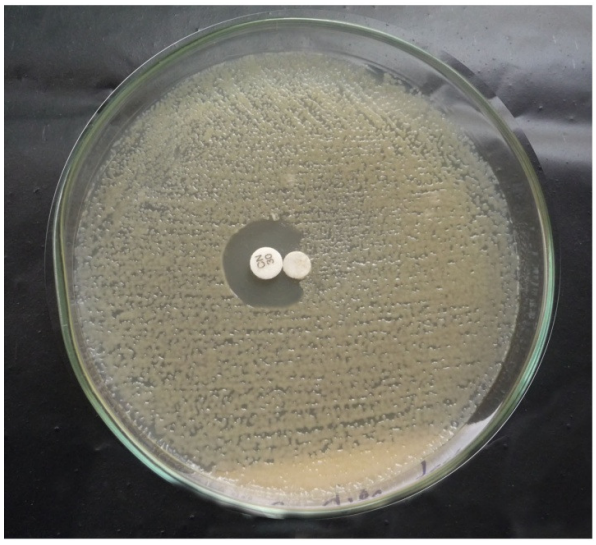
Klebsiella pnemoniae ATCC 700603 – Positive control

Tests for Amp C β lactamase production

Disc Antagonism test[92]



The organisms that exhibited resistance to 3rd generation cephalosporins and cefoxitins were swabbed onto a Muller - Hinton Agar Plate and Cefoxitin (30 µg) and Ceftaxidime (30 µg) discs are placed at a distance of 20mm from centre to centre and incubated overnight at 37° C. Amp C β-lactamases inducibility was recognized by blunting of the Ceftazidime zone adjacent to Cefoxitin disc[92,93].



Amp C disc test (Black *et al.*, 2005)[94]

The test is based on the use of Tris – EDTA to permeabilize bacterial cell and release β-lactamases into the external environment. Amp C discs (i.e., filter paper disks containing Tris-EDTA) were prepared in house by applying 20 µl

of 1:1 mixture of saline and 100 X Tris – EDTA to sterile filter paper discs allowing the discs to dry and storing them at 2- 8 °C. The surface of a MHA plate was inoculated with a lawn of Cefoxitin- susceptible *E. coli* ATCC 25922 according to the standard disc diffusion method. Immediately prior to use, Amp C discs were rehydrated with 20 µl of saline and several colonies of each test organism were applied to a disc.

A 30 µg Cefoxitin disc was placed on the inoculated surface of the MHA. The inoculated Amp C disc was then placed almost touching the antibiotic disc with the inoculated disc face in contact with the Agar surface. The plate was then inverted and incubated overnight at 35 °C in ambient air. After incubation, plates were examined for either a distortion, indicating no significant inactivation of Cefoxitin (positive result), or the absence of a distortion, indicating no significant inactivation of Cefoxitin (negative result).

LIMITATIONS OF THE STUDY :

1. Isolation of Anaerobes was not done.
2. Genomic study was not carried out.

STATISTICS: Simple descriptive statistics was used to analyze the data.

TABLE 6: Zone Size Interpretation Chart According To CLSI[87]

Sl. No.	Antimicrobial agent	Symbol	Drug concentration (µg)	Zone size in mm		
				Resistant	Intermediate	Sensitive
A. Aminoglycosides						
1.	Gentamicin	G	10	< 12	13-14	> 15
2.	Amikacin	AK	30	<14	15-16	>17
B. Penicillin						
1.	Ampicillin	A	10	<13	14-16	>17
C. Sulphonamides						
1.	Cotrimoxazole	CO	1.25/23.75	<10	11-15	>16
D. Quinolones						
1.	Nalidixic acid	NA	30	<13	14-13	>19
2.	Norfloxacin	NX	10	<12	13-16	>17
3.	Ciprofloxacin	CF	5	<15	16-20	>21
4.	Levofloxacin	LE	5	<13	14-16	>17
E. Cephalosporins						
1.	Cephelexin	CP	30	<14	15-17	>18
2.	Cefuroxime	CU	30	<14	15-17	>18
3.	Cefoxitin	CN	30	<14	15-17	>18
4.	Ceftazidime	CA	30	<14	15-17	>18
5.	Ceftriaxone	CI	30	<13	14-20	>21
6.	Cefotaxime	CE	30	<14	15-22	>23
7.	Cefpodoxime	CEP	10	<17	18-20	>21
8.	Cefipime	CPM	30	<14	15-17	>18
F. Monobactams						
1.	Aztreonam	AO	30	<15	16-21	>22
G. Carbapenems						
1.	Imipenam	I	10	<13	14-15	>16
H. β lactam - β lactamase inhibitor						
1	Amoxyclav	AC	20/10	<13	14 – 17	>18
2	Piperacillin-Tazobactum	PT	100/10	17	18 – 20	21
3	Cefeperazone-Sulbactum	CFS	75 / 10	15	16 20	21
I. Macrolides						
1.	Azithromycin	AT	15	<13	14-17	>18
K. Glycopeptide						
1.	Vancomycin	V	30	<14	----	>15

RESULTS

During the study period from April 2011 to April 2012, a total number of 142 samples were collected from 142 patients with DFI/DFU attending SURGERY and DIABETOLOGY Out Patient Department and those admitted at Thanjavur Medical College Hospital, Thanjavur. The total 142 samples were processed in the Microbiology laboratory of Thanjavur Medical College and Hospital.

Among the total population, 85 males (59.85%) and 57 females (40.15%) were affected by Diabetic foot infection [Table 1]. The mean age of the subjects was Out of the 142 specimens processed, 119 (84%) showed significant growth and 23 (16%) yielded no growth of organisms.

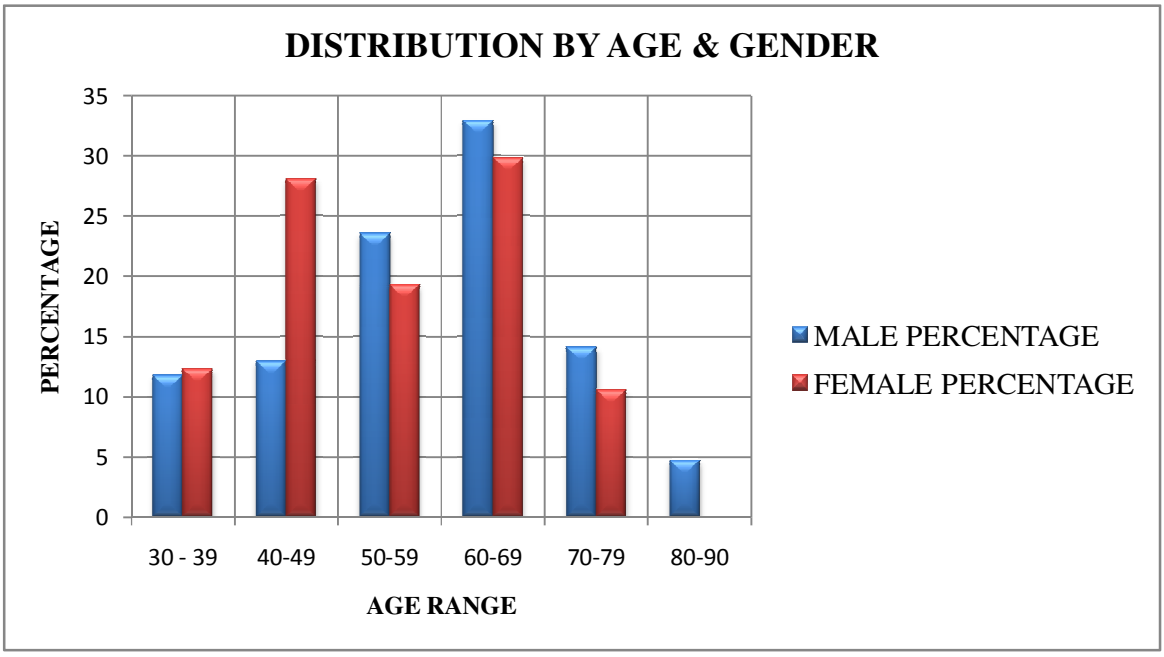
The processed 119 samples yielded a total of 165 organisms, the polymicrobial isolation being the reason behind. 81(68%) samples yielded monomicrobial growth while 38 samples (32%) showed polymicrobial growth.

The following organisms were isolated from the 142 samples subjected for study and the 119 positive cultures. Staphylococcus aureus - 31(26%), Pseudomonas spp. - 28(24%), Proteus spp.- 25(21%), Enterococci -15(13%) Enterobacter-14(12%), Klebsiella spp. - 13(11%), Escherichia coli - 10(8%), CONS - 10(8%), Corynebacterium spp.-8(7%), Nonhemolytic Streptococci-5(4%), Acinetobacter-3 (2.5%) and Citrobacter - 3(2.5%) [Table 4].

TABLE 7: Distribution by Age & Gender

DISTRIBUTION BY AGE & GENDER						
AGE RANGE	MALE		FEMALE		TOTAL	PERCENTAGE
	No.	PERCENTAGE	No.	PERCENTAGE		
30 – 39	10	12	7	12	17	12
40-49	11	13	16	28	27	19
50-59	20	24	11	19	31	22
60-69	28	33	17	30	45	32
70-79	12	14	6	11	18	13
80-90	4	5	0	0	4	3

CHART 1: **Distribution by Age & Gender**

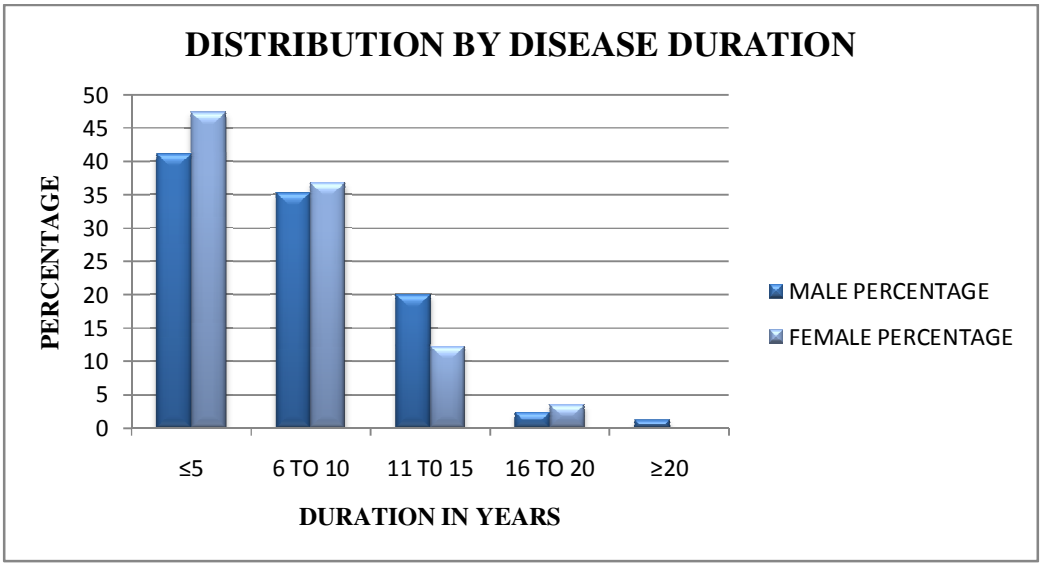


The prevalence of DFI was more in males than females. Majority of the study subjects fall in the 60 – 69 age group.

TABLE 8: Distribution by Disease Duration

DISTRIBUTION BY DISEASE DURATION						
DURATION IN YEARS	MALE		FEMALE		TOTAL	PERCENTAGE
	NO.	PERCENTAGE	NO.	PERCENTAGE		
≤5	35	41	27	47	62	44
6 TO 10	30	35	21	37	51	36
11 T0 15	17	20	7	12	24	17
16 TO 20	2	2	2	4	4	3
≥20	1	1	0	0	1	1
	85		57		142	100

CHART 2: Distribution by Disease Duration

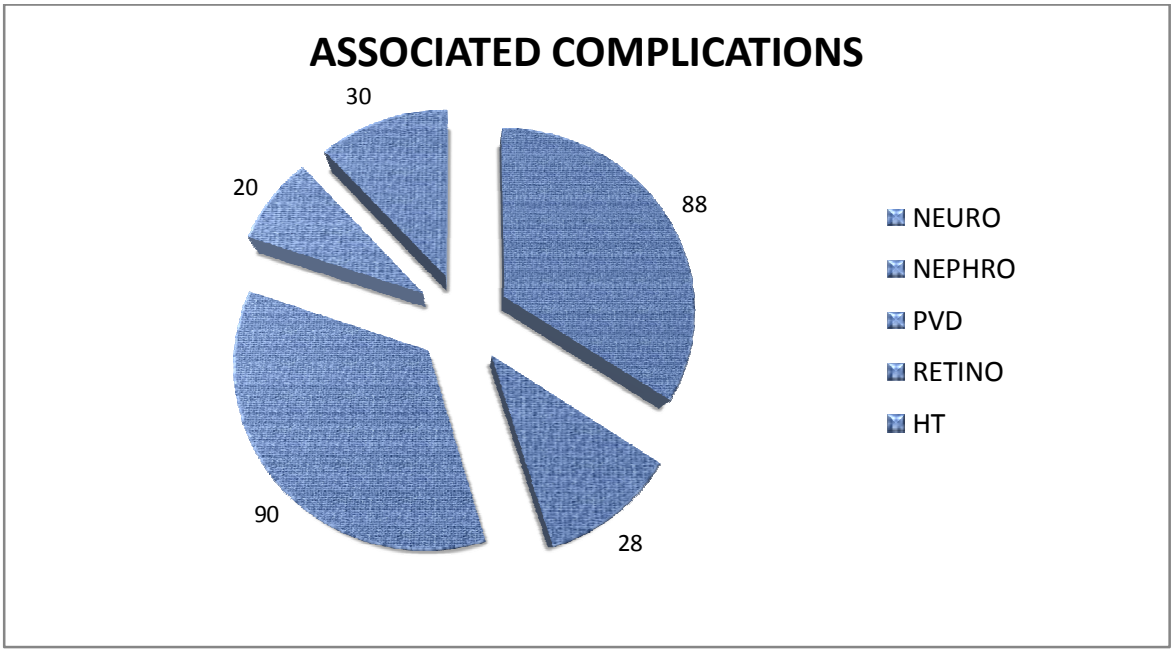


80% of the study subjects were suffering from diabetes for a period of about 10 years. Greater the duration of illness higher the incidence of MDRO isolates.

TABLE 9: Incidence of Associated Complications

ASSOCIATED COMPLICATIONS		
DISEASE	**NUMBER OF PATIENTS**	**PERCENTAGE**
NEUROPATHY	125	88
NEPHROPATHY	40	28
PERIPHERAL VASCULAR DISEASE	127	90
RETINOPATHY	29	20
HYPERTENSION	43	30

CHART 3: Incidence of Associated Complications

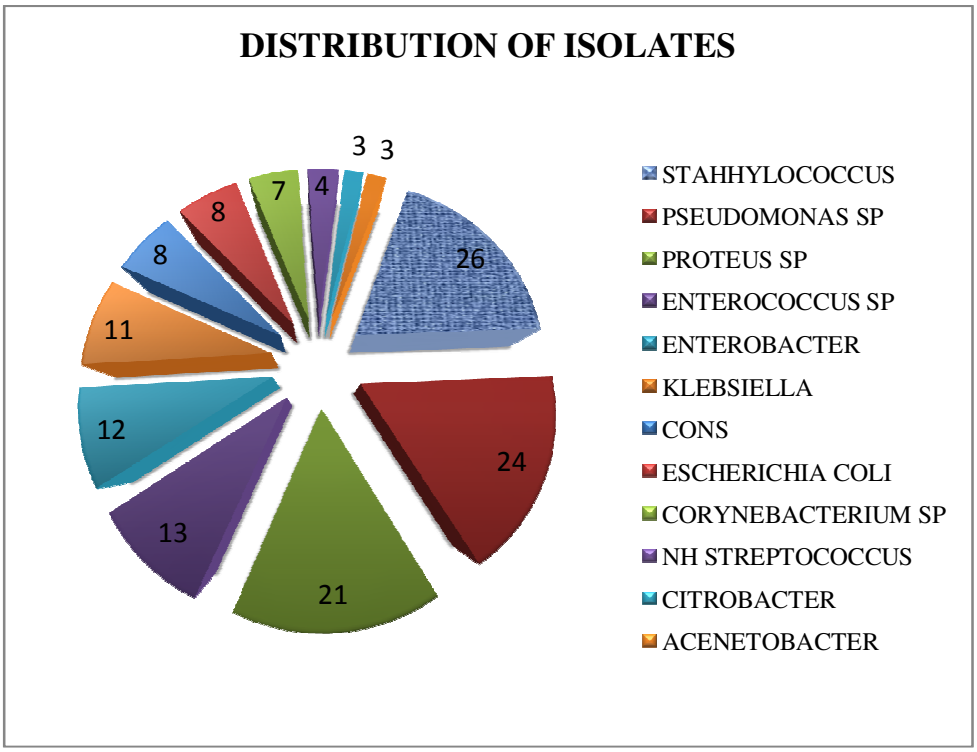


90% of the patients had Peripheral vascular disease and 88% of the study population had neuropathy as associated complication of the disease and as risk factor for development of ulcer.

TABLE 10: Distribution of Isolates

S.NO	NAME OF THE ISOLATE	NO.ISOLATED	PERCENTAGE
1	STAHHYLOCOCCUS	31	26
2	PSEUDOMONAS SP	28	24
3	PROTEUS SP	25	21
4	ENTEROCOCCUS SP	15	13
5	ENTEROBACTER	14	12
6	KLEBSIELLA	13	11
7	CONS	10	8
8	ESCHERICHIA COLI	10	8
9	CORYNEBACTERIUM SP	8	7
10	NH STREPTOCOCCUS	5	4
11	CITROBACTER	3	3
12	ACENETOBACTER	3	3
		165	

CHART 4: Distribution of Isolates



58% of isolates are Aerobic gram negative organisms and remaining 42% being aerobic gram positive bacteria.38 out of 119 patients showed polymicrobial growth.

Staphylococcus is the most frequent pathogen found in nearly 26% of infection. Enterococcus Spp. constitutes next frequent gram positive isolate. (13%). Majority of studies also noted high frequency of these microorganisms in foot infection of diabetic patients. [39, 41, 42]

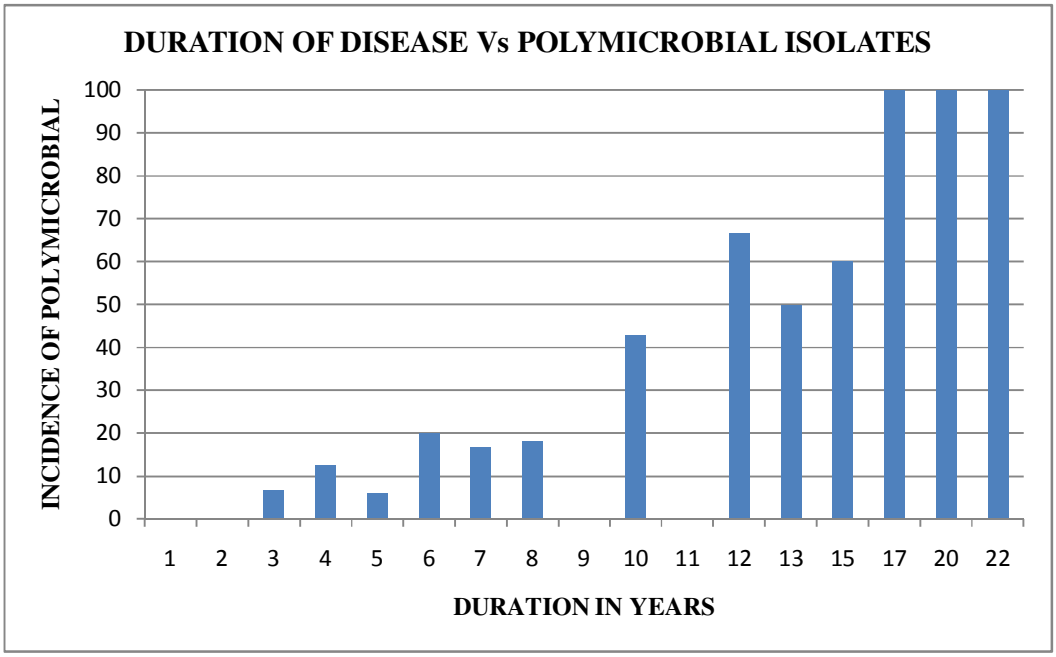
In this study gram negative bacteria were most frequently isolated, this finding is in concurrence with the several other Indian studies [40, 42,43] though the same is in contrast with the foreign studies. The difference in age - sex composition, study setting, socio economic factors might be reason behind these differences.

There was high recovery of pseudomonas isolates in these study subjects that showed multi drug resistance as well. This raises a serious concern as multi drug resistant isolates were more frequently found in hospitalized patients after considerable period of stay. The usage of broad spectrum antibiotics, prolonged usage of antibiotics could be the factors behind this observation. [42]

TABLE 11: Correlation between Duration of Disease, Polymicrobial Isolates and MDRO Occurrences

INCIDENCE OF MDRO & POLYMICROBIAL					
Duration of Diabetes in years	**Total number of Patients**	**Total number of MDRO**	**MDRO Percentage**	**Total number of Polymicrobial**	**Polymicrobial Percentage**
1	4	3	75	0	0
2	18	8	44	4	22
3	15	7	47	4	27
4	8	4	50	1	13
5	17	8	47	10	59
6	5	2	40	3	60
7	6	5	83	3	50
8	11	6	55	5	45
9	1	1	100	1	100
10	28	21	75	20	72
11	1	1	100	1	100
12	6	3	50	4	67
13	2	2	100	2	100
15	15	10	67	12	80
17	1	1	100	1	100
20	3	2	67	2	67
22	1	1	100	1	100

CHART 5: Correlation between Duration of Disease and Polymicrobial Isolates



As the duration of the disease increases the frequency of polymicrobial colonization and MDRO incidence is also more.This observation is explaind by the immunological compromise experienced by diabetics and prolonged periods of treatment. This is supported by studies from northern India. (43)

The prolonged periods of hospital stay also increases the incidence of polymicrobial infection and development of multi drug resistance. This is in accordance with the other studies.(43,44)

CHART 6: Correlation between Duration of Disease and MDRO Isolates

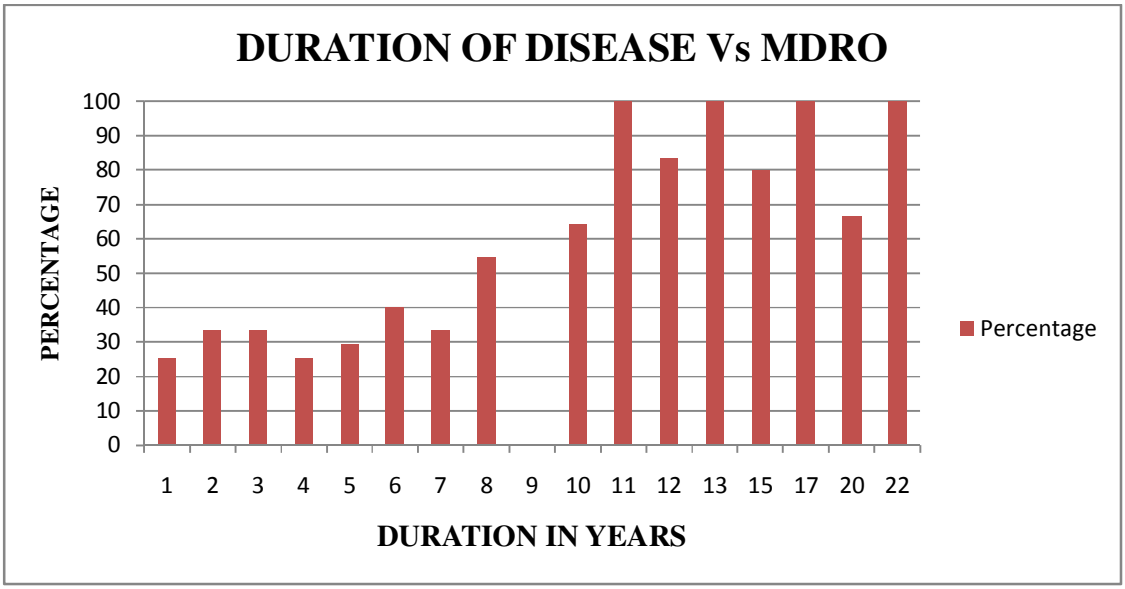


TABLE 12: Relationship between Prolonged Stay, MDRO & Polymicrobial Isolates

RELATIONSHIP BETWEEN DURATION OF STAY & INCIDENCE OF MDRO					
Stay in Weeks	**Total Number of Patients**	**Total Number of MDRO**	**MDRO Percentage**	**Total number of Polymicrobial**	**Polymicrobial Percentage**
1	5	3	60	0	0
2	25	15	60	2	8
3	30	7	23	5	17
4	24	10	42	4	17
5	10	6	60	4	40
6	27	17	63	10	37
7	1	1	100	1	100
8	12	10	83	10	83
12	5	5	100	5	100

CHART 7: Relationship between Prolonged Stay & MDRO Isolates

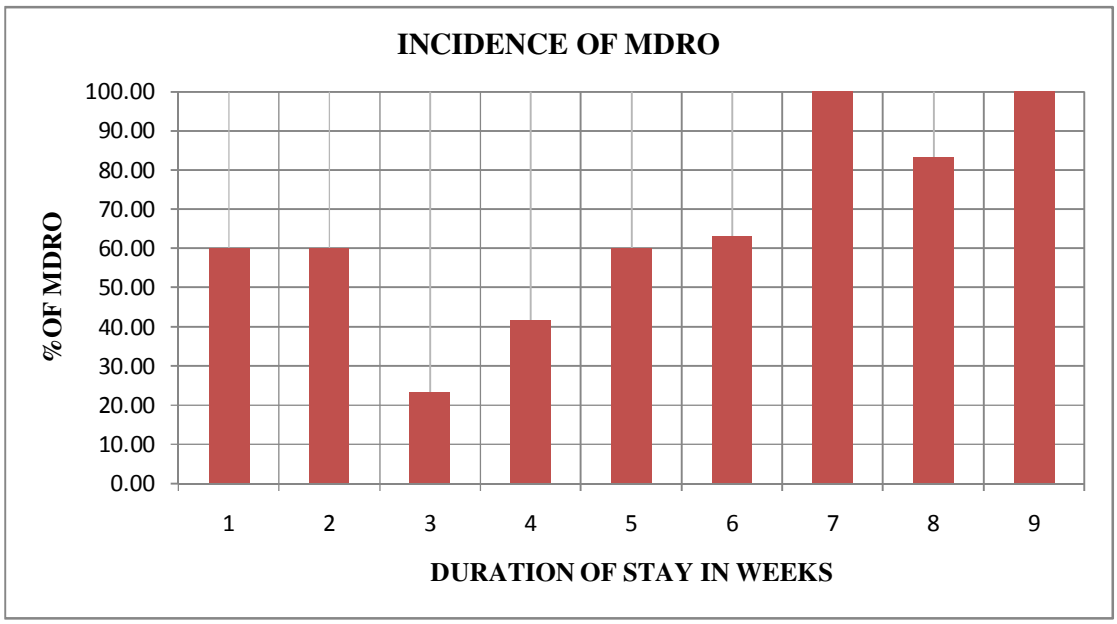


CHART 8: Relationship between Prolonged Stay & Polymicrobial Isolates

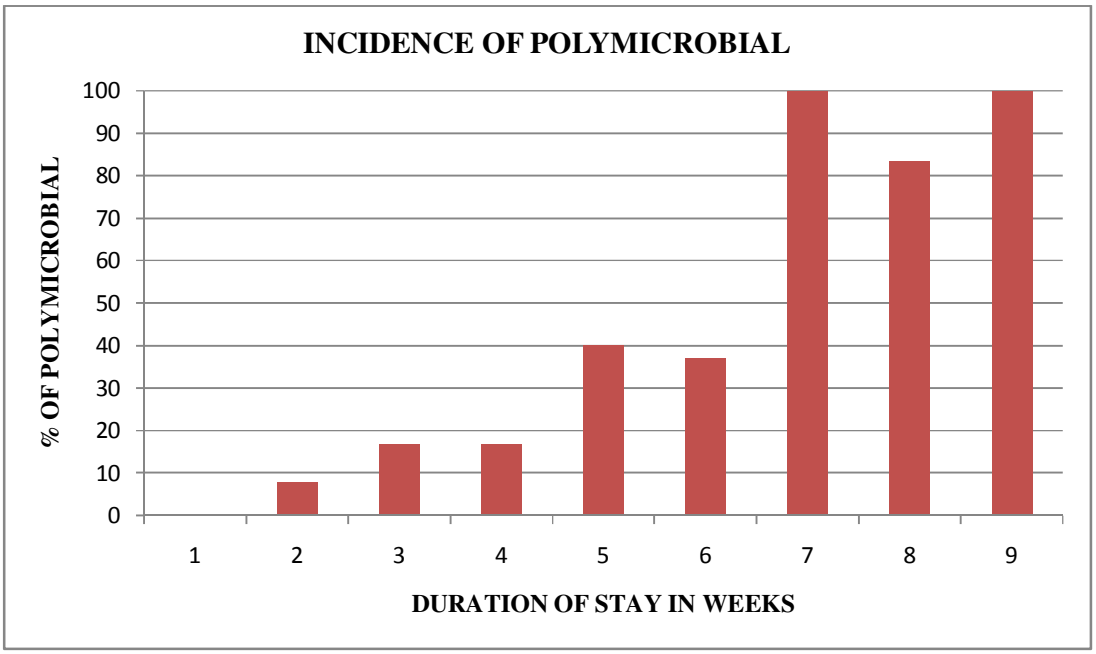


TABLE 13: Correlation between Glycemic Control & Amputation

Glycemic control	Total	MDRO		Polymicrobial infection		Amputation	
		No.	Percentage	No.	Percentage	No.	Percentage
FBS≥110	98	55	56	25	26	87	96
PPBS≥160	142	71	50	29	30	91	100

Poor glycemic control in patients is identified as an independent risk factor for MDRO infection and in turn to amputation that is in accordance with a study in AIIMS.(43)

Amputation rates are higher when there is infection with multi drug resistant organism. Among the patients with MDRO infection 96% of them underwent amputation.62% of total amputations were performed on patients with MDRO infection.

CHART 9: Correlation between Glycemic Control & Amputation

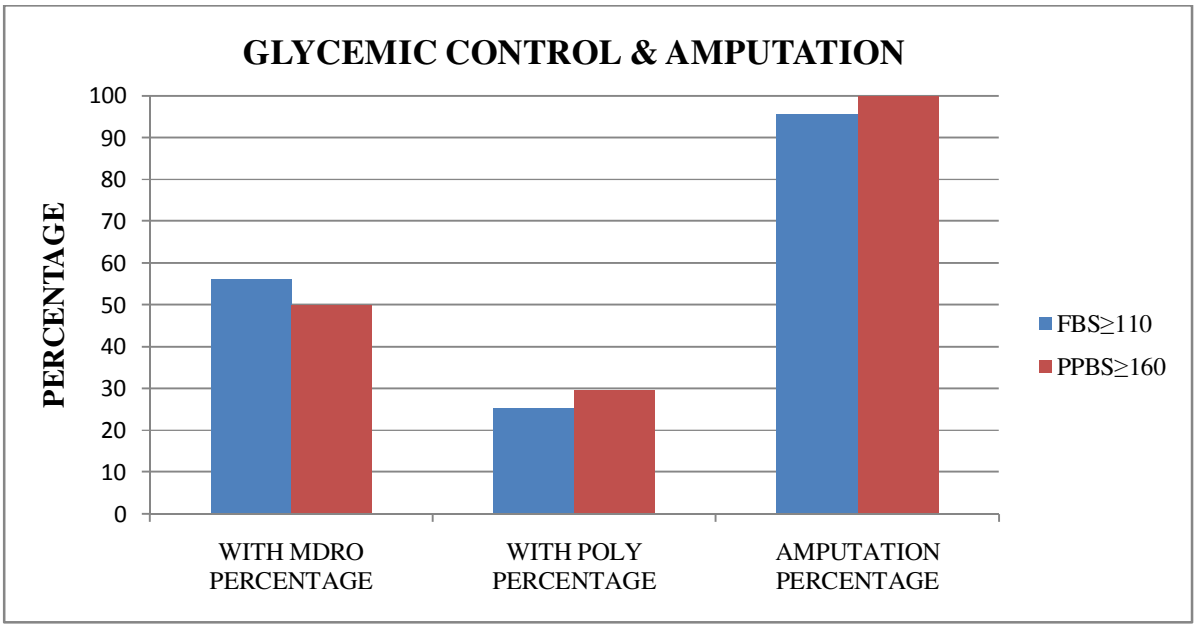


TABLE 14: Clinical Outcome

	CONSERVATIVE	AMPUTATION
TOTAL PATIENTS	51	91
PERCENTAGE	**36%**	**64%**

CHART 10: Clinical Outcome

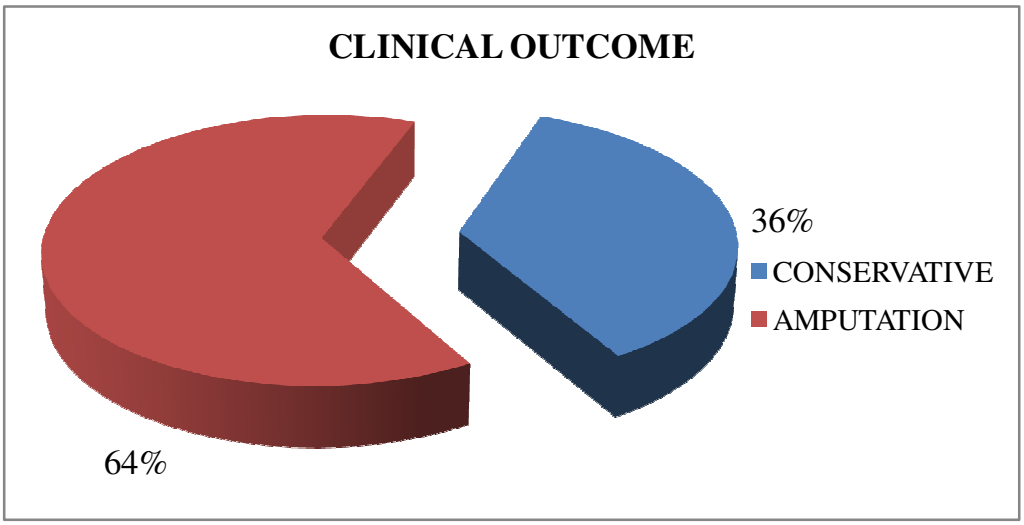


TABLE15: Correlation between MDRO & Amputation

	AMPUTATION	MDRO	POLYMICROBIAL GROWTH
NO.OF PATIENTS	91	56	24
PERCENTAGE	**64%**	**62%**	**26.37%**

TABLE16: AMS Pattern of STAPHYLOCOCCUS

STAPHYLOCOCCUS AUREUS (N =31)					
Sl.no	DRUG	RESISTANCE		SUSCEPTIBLE	
		No.	PERCENTAGE	No.	PERCENTAGE
1	Ampicillin	31	100.00	0	0.00
2	Amoxycillin	31	100.00	0	0.00
3	Amoxyclav	0	0.00	31	100.00
4	Oxacillin	8	25.81	23	74.19
5	Erythromycin	0	0.00	31	100.00
4	Azithromycin	0	0.00	31	100.00
7	Cotrimoxazole	0	0.00	31	100.00
8	Doxycycline	0	0.00	31	100.00
9	Amikacin	0	0.00	31	100.00
10	Gentamicin	0	0.00	31	100.00
11	Ciprofloxacin	0	0.00	31	100.00
12	Ofloxacin	0	0.00	31	100.00
13	Cephelexin	0	0.00	31	100.00
14	Cefixime	31	100.00	0	0.00
15	Cefotaxime	8	25.81	23	74.19
16	Ceftrioxone	8	25.81	23	74.19
17	Ceftazidime	28	90.32	3	9.68
18	Ceftazidime+clav	31	100.00	0	0.00
19	Cefipime	5	16.13	26	83.87
20	Piperacillin+Tazo	0	0.00	31	100.00
21	Aztreonam	8	25.81	23	74.19
22	Imipenam	0	0.00	31	100.00
23	Vancomycin	0	0.00	31	100.00

MRSA constitutes 26% of the total Staphylococcal isolates.Multidrug resistance was a common feature observed among the organisms.

CHART 11: AMS Pattern of STAPHYLOCOCCUS

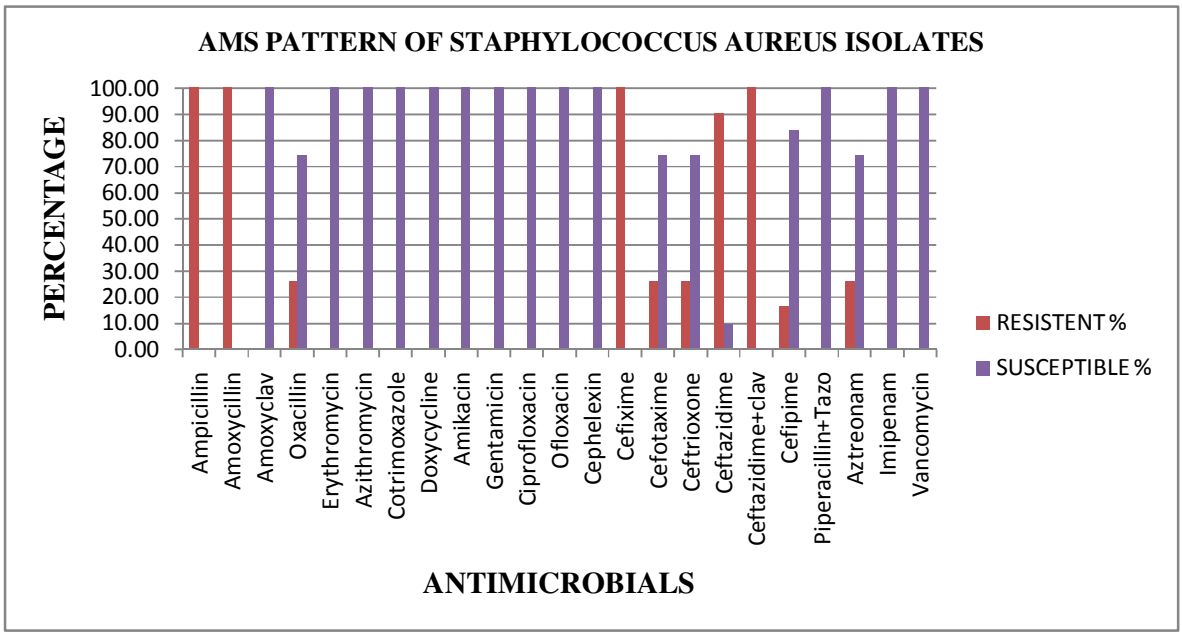


CHART 12 : AMS PATTERN OF PSEUDOMONAS ISOLATES

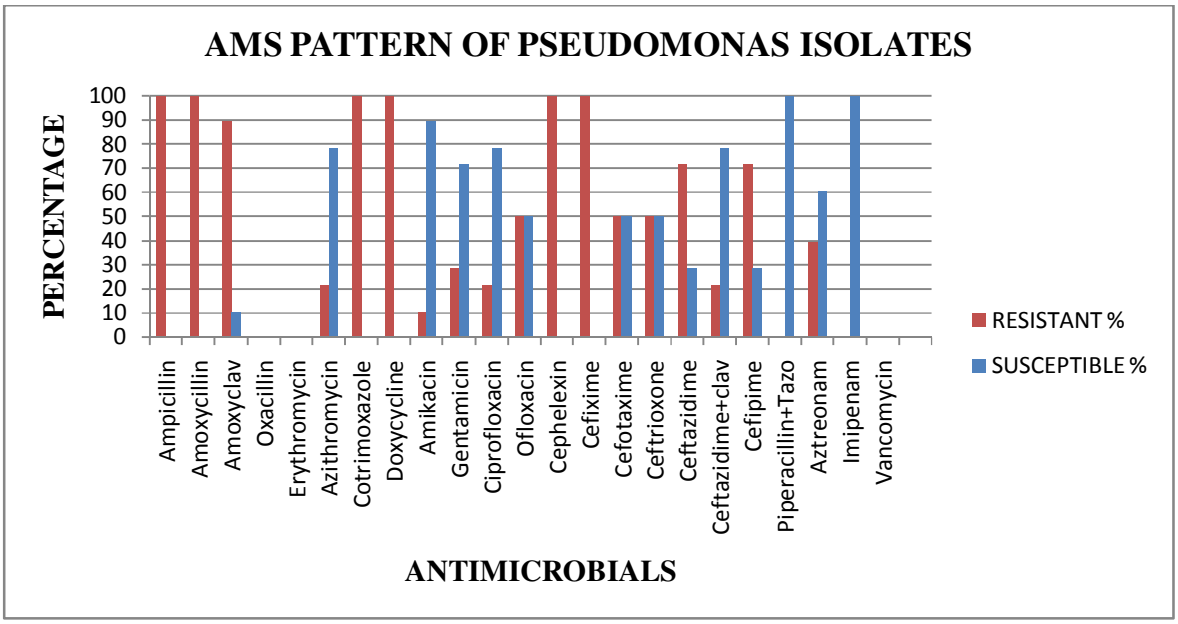


TABLE17: AMS PATTERN OF PSEUDOMONAS

PSEUDOMONAS (N=28)					
Sl.NO.	DRUG	RESISTANT		SUSCEPTIBLE	
		No.	PERCENTAGE	No.	PERCENTAGE
1	Ampicillin	28	100	0	0
2	Amoxycillin	28	100	0	0
3	Amoxyclav	25	89.28	3	10.71
4	Oxacillin	0	0	0	0
5	Erythromycin	0	0	0	0
4	Azithromycin	6	21.42	22	78.57
7	Cotrimoxazole	28	100	0	0
8	Doxycycline	28	100	0	0
9	Amikacin	3	10.71	25	89.28
10	Gentamicin	8	28.57	20	71.42
11	Ciprofloxacin	6	21.42	22	78.57
12	Ofloxacin	14	50	14	50
13	Cephelexin	28	100	0	0
14	Cefixime	28	100	0	0
15	Cefotaxime	14	50	14	50
16	Ceftrioxone	14	50	14	50
17	Ceftazidime	20	71.42	8	28.57
18	Ceftazidime+clav	6	21.42	22	78.57
19	Cefipime	20	71.42	8	28.57
20	Piperacillin+Tazo	0	0	28	100
21	Aztreonam	11	39.28	17	60.71
22	Imipenam	0	0	28	100
23	Vancomycin	0	0	0	0

All the isolates are resistant to Ampicillin, Amoxycillin, Cotrimoxazole, Doxycycline, Cephelexin and Cefixime while all are susceptible to Piperacillin-Tazobactum and Imipenam. Prevalence of ESBL producers is 21.5%.

TABLE18: AMS PATTERN OF KLEBSIELLA

KLEBSIELLA(N=13)					
Sl.NO.	DRUG	RESISTANT		SUSCEPTIBLE	
		No.	PERCENTAGE		PERCENTAGE
1	Ampicillin	13	100.00	0	0.00
2	Amoxycillin	13	100.00	0	0.00
3	Amoxyclav	9	69.23	4	30.77
4	Oxacillin	0	0.00	0	0.00
5	Erythromycin	0	0.00	0	0.00
4	Azithromycin	0	0.00	13	100.00
7	Cotrimoxazole	9	69.23	4	30.77
8	Doxycycline	4	30.77	9	69.23
9	Amikacin	0	0.00	13	100.00
10	Gentamicin	0	0.00	13	100.00
11	Ciprofloxacin	4	30.77	9	69.23
12	Ofloxacin	4	30.77	9	69.23
13	Cephelexin	4	30.77	9	69.23
14	Cefixime	13	100.00		0.00
15	Cefotaxime	4	30.77	9	69.23
16	Ceftrioxone	4	30.77	9	69.23
17	Ceftazidime	8	61.54	5	38.46
18	Ceftazidime+clav	4	30.77	9	69.23
19	Cefipime	4	30.77	9	69.23
20	Piperacillin+Tazo	0	0.00	13	100.00
21	Aztreonam	4	30.77	9	69.23
22	Imipenam		0.00	13	100.00
23	Vancomycin	0	0.00	0	0.00

All the isolates are resistant to Ampicillin, Amoxycillin and Cefixime while all are susceptible to Aminoglycosides, Aithromycin, Piperacillin-Tazobactum, and Imipenam. Prevalence of ESBL producers is 31%.

CHART 13 : AMS PATTERN OF KLEBSIELLA ISOLATES

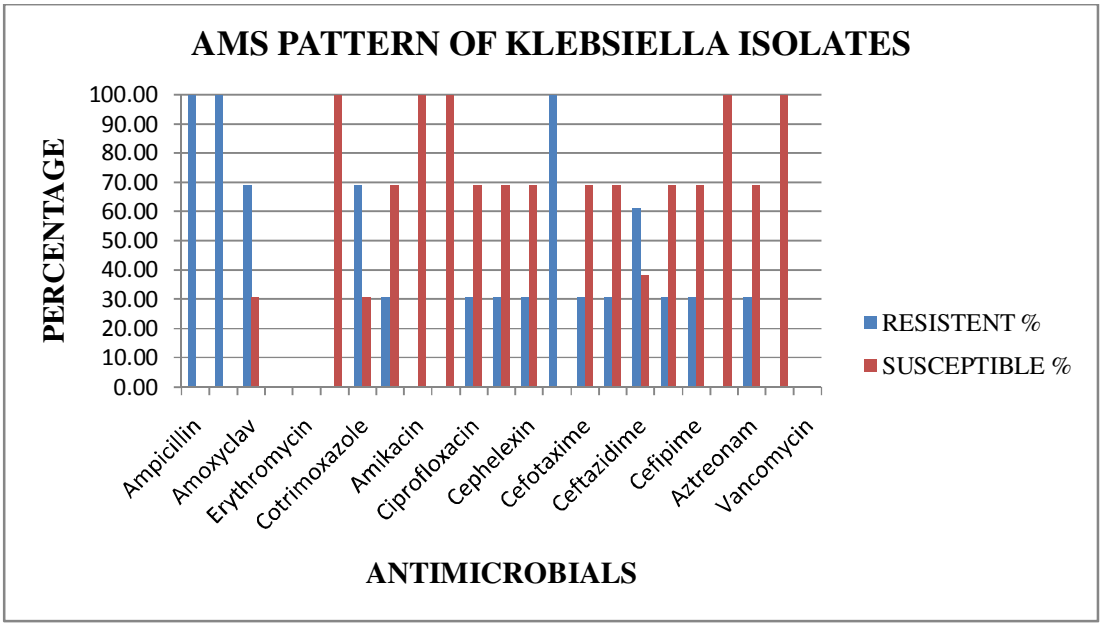


CHART 14: AMS PATTERN OF ENTEROBACTER

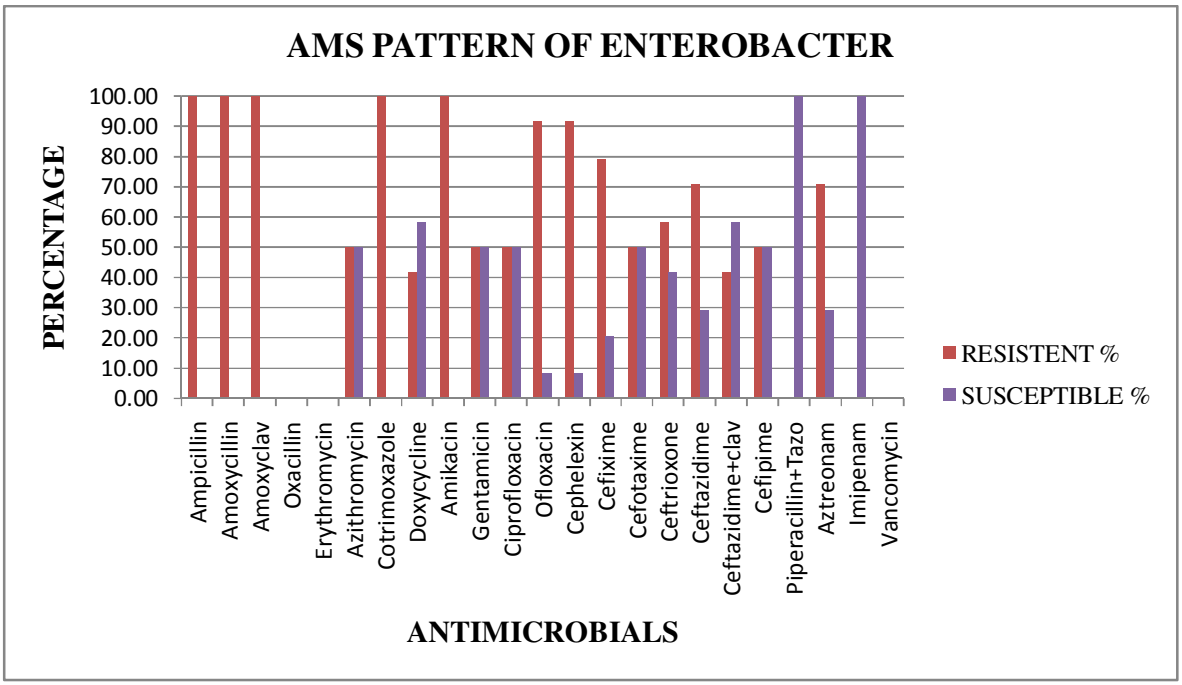


TABLE19: AMS PATTERN OF ENTEROBACTER

ENTEROBACTER(N=24)					
Sl.NO	DRUG	RESISTANT		SUSCEPTIBLE	
		No.	PERCENTAGE		PERCENTAGE
1	Ampicillin	24	100.00	0	0.00
2	Amoxycillin	24	100.00	0	0.00
3	Amoxyclav	24	100.00	0	0.00
4	Oxacillin	0	0.00	0	0.00
5	Erythromycin	0	0.00	0	0.00
4	Azithromycin	12	50.00	12	50.00
7	Cotrimoxazole	24	100.00	0	0.00
8	Doxycycline	10	41.67	14	58.33
9	Amikacin	24	100.00	0	0.00
10	Gentamicin	12	50.00	12	50.00
11	Ciprofloxacin	12	50.00	12	50.00
12	Ofloxacin	22	91.67	2	8.33
13	Cephelexin	22	91.67	2	8.33
14	Cefixime	19	79.17	5	20.83
15	Cefotaxime	12	50.00	12	50.00
16	Ceftrioxone	14	58.33	10	41.67
17	Ceftazidime	17	70.83	7	29.17
18	Ceftazidime+clav	10	41.67	14	58.33
19	Cefipime	12	50.00	12	50.00
20	Piperacillin+Tazo	0	0.00	24	100.00
21	Aztreonam	17	70.83	7	29.17
22	Imipenam	0	0.00	24	100.00
23	Vancomycin	0	0.00	0	0.00

All the isolates are resistant to Ampicillin, Amoxycillin, Amoxyclav, Cotrimoxazole and Amikacin .All are susceptible to Piperacillin-Tazobactum, and Imipenam. Prevalence of ESBL producers is 42%.

TABLE 20: AMS PATTERN OF PROTEUS

PROTEUS N=25					
Sl.NO	DRUG	RESISTANT		SUSCEPTIBLE	
		No.	PERCENTAGE		PERCENTAGE
1	Ampicillin	16	64	9	36
2	Amoxycillin	16	64	9	36
3	Amoxyclav	12	48	13	52
4	Oxacillin	0	0	0	0
5	Erythromycin	0	0	0	0
4	Azithromycin	3	12	22	88
7	Cotrimoxazole	16	64	9	36
8	Doxycycline	3	12	22	88
9	Amikacin	0	0	25	100
10	Gentamicin	0	0	25	100
11	Ciprofloxacin	3	12	22	88
12	Ofloxacin	3	12	22	88
13	Cephelexin	6	24	19	76
14	Cefixime	13	52	12	48
15	Cefotaxime	3	12	22	88
16	Ceftrioxone	3	12	22	88
17	Ceftazidime	3	12	22	88
18	Ceftazidime+clav	3	12	25	100
19	Cefipime	3	12	22	88
20	Piperacillin+Tazo	0	0	25	100
21	Aztreonam	3	12	22	88
22	Imipenam	0	0	25	100
23	Vancomycin	0	0	0	0

100% sensitivity was observed for Aminoglycosides, Ceftazidime – Clavulanic acid, Piperacillin – Tazobactum and Imipenam.64% of the organisms were resistant to Ampicillin and Amoxycillin.

CHART 15: AMS PATTERN OF PROTEUS

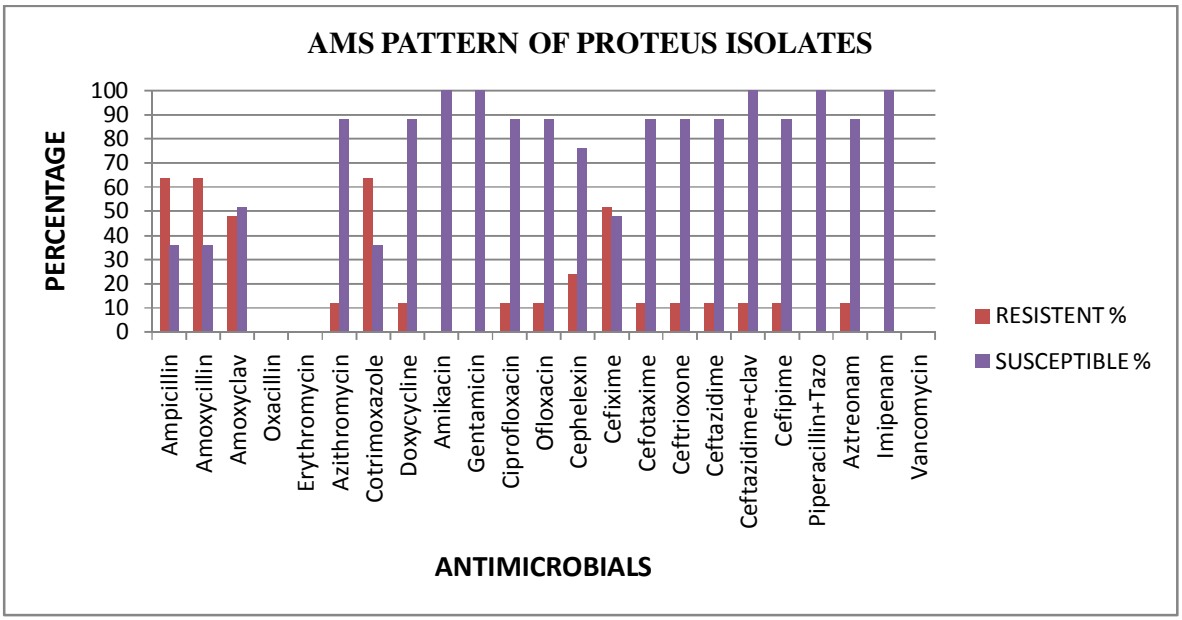


CHART 16: AMS PATTERN OF ESCHERICHIA COLI

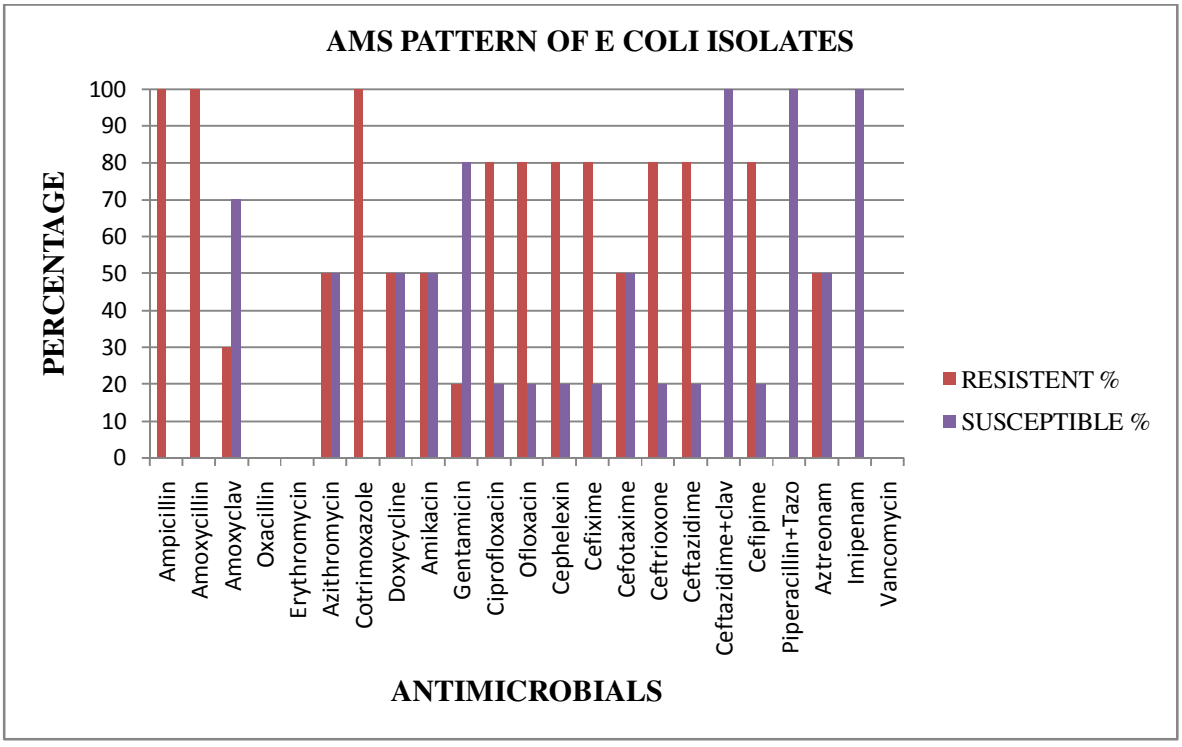


TABLE 21: AMS PATTERN OF E COLI

E COLI N=10					
Sl.NO	DRUG	RESISTANT		SUSCEPTIBLE	
		No.	PERCENTAGE	No.	PERCENTAGE
1	Ampicillin	10	100	0	0
2	Amoxycillin	10	100	0	0
3	Amoxyclav	3	30	7	70
4	Oxacillin	0	0	0	0
5	Erythromycin	0	0	0	0
4	Azithromycin	5	50	5	50
7	Cotrimoxazole	10	100	0	0
8	Doxycycline	5	50	5	50
9	Amikacin	5	50	5	50
10	Gentamicin	2	20	8	80
11	Ciprofloxacin	8	80	2	20
12	Ofloxacin	8	80	2	20
13	Cephelexin	8	80	2	20
14	Cefixime	8	80	2	20
15	Cefotaxime	5	50	5	50
16	Ceftrioxone	8	80	2	20
17	Ceftazidime	8	80	2	20
18	Ceftazidime+clav	6	60	4	40
19	Cefipime	8	80	2	20
20	Piperacillin+Tazo	0	0	10	100
21	Aztreonam	5	50	5	50
22	Imipenam	0	0	10	100
23	Vancomycin		0		0

All the isolates are resistant to Ampicillin, Amoxycillin and Cotrimoxazole while all are susceptible to Piperacillin-Tazobactum and Imipenam. Prevalence of ESBL producers is 60%.

DISCUSSION

As the domestic and international incidence of diabetes and its related complications continues to rise, medical fraternity needs to continue to improvise the management of the same. Since the burden on socioeconomic aspects is on the ascending mode, a like-minded multidisciplinary team approach is needed to optimize the patient care. Early recognition of severe infections in addition to other modalities of management is a crucial component of managing diabetic foot infections.

AGE AND SEX WISE DISTRIBUTION:

In this study the incidence of the disease is more among men than women as supported by various international and national data[95]. Among the total 142 patients 59.85% constitutes male population, the remaining 40.15% being the female population.

The mean age of the subjects was 56.75years. Older population falling in the age group of 60 – 69 years contributes the majority (32%) of the diseased undergoing treatment for DFI in our hospital. This may be attributed to lesser survival rate among above-70 age group in our country which in turn puts the blame on socioeconomic factors.

DISEASE DURATION:

The mean duration of Diabetes was 7.58 years and more than half of the population (56%) had the disease for ≥5years.This may be explained by the increasing awareness about the illness among people. Prolonged duration of illness and hence long periods of hyperglycemia predisposes to MDRO incidence. This is in harmony with the Observations by Ravishankar Gadepalli et al in a field study in North India.[97,98]

ASSOCIATED COMPLICATIONS:

The incidence of other diabetes related complications in patients presented with DFI is as follows – 125 among 142 i.e.88% of the patients presented with Neuropathy. Various studies all over the world[3, 14,98,99,100,101] including The IDSA guideline support and confirm this finding. Peripheral vascular disease was observed in 89% of the patients. Retinopathy – 29(20%), Hypertension - 43(30%). Nephropathy – 40 (28%) were the other diabetes related comorbidities observed. There is a positive correlation between the incidence of MDRO and peripheral vascular disease which is supported by other studies.

PROFILE OF PATHOGENS IN DFI:

Of the 142 cases studied 23 did not yield any pathogen on culture which may be attributed to prior antibiotic therapy, organism's less than critical level colonization or unfavourable growth conditions (Anaerobes were not included in this study).

119 specimens yielded bacterial growth of which 38 showed polymicrobial isolates. A total of 165 aerobic bacteria were isolated, 96 (58%) being Gram negative, 69 (42%) being Gram positive, giving the majority to Gram negative bacilli. This is in contrast to Western studies[36, 37, 38, and 39] but in concurrence with national scenario[40].

Staphylococcus aureus ranks first among the bacterial pathogens amounting to 26% of the isolates which correlates with national and Global occurrence[39, 41, 42]. Pseudomonas aeruginosa follows with 23.5% of incidence[42]. Proteus (21%), Enterococcus sp (13%), Enterobacter (12%), Klebsiella (11%), CONS & E.coli (8.4%) are the other significant isolates that worth mention here.

POLYMICROBIAL NATURE:

In this study incidence of polymicrobial isolates in chronic DFI is around 27 % i.e. from 38 out of 142 samples two to three organisms were isolated. Further it is influenced by the duration of illness and hospital stay i.e.36 out of 38 (94.7%) polymicrobial growth was observed in subjects with illness for ≥5 years. The inherent susceptibility to infection in diabetics attributed to impaired leukocyte chemotaxis and phagocytosis is exhibited by this polymicrobial isolation. 37 out of 38 (97.3%) patients showing polymicrobial growth stayed in the hospital for ≥4weeks. The contamination and colonization by hospital flora is the obvious factor behind hence needs no explanation. These findings correlate with South Indian, North Indian and global data.[97,98]

MULTIDRUG RESISTACE:

The MDR isolates among the total isolates constitutes 43% i.e.71 out of 165 isolates. Patients who stayed in the hospital for ≥ 4weeks harboured 70.4% of the MDR isolates. Increasing prevalence of multidrug resistance among hospital flora explains this scenario. Patients with prolonged duration of illness showed increased isolation of MDR organisms amounting to 80.3% of total MDRO isolated in this study. Molecular level insults done by prolonged hyperglycemia compromising the vascularity and hence the achievement of therapeutic index of antimicrobials of MDRO in the community itself. Retrospective analysis shows MDRO infected patients had poor glycemic control during the course of illness. This is in harmony with AIIMS study by Ravisheher et al.Similarly coexistent Hypertension also predisposes to MDRO infection. Rational usage of antimicrobials based on the local prevalence of organisms and their sensitivity pattern is the need of the hour. Further research aimed in this direction to obtain and document the local microbial flora and resistance scenario is essential.

AMS PATTERN OF STAPHYLOCOCCUS AUREUS:

Staphylococcus aureus is the commonest aerobic bacteria isolated in this study. This is in harmony with various studies in different localities of the world.[96]The AMS profile of the organism showed 74% of MSSA and 26% of MRSA. Irrespective of the Methicillin susceptibility status, invariably all the Staphylococcal isolates showed resistance to Ampicillin, Amoxycillin, Cefixime and Ceftazidime-Clavulanic acid combination. The isolates resistant to Ceftazidime-Clavulanic acid combination are all invariably sensitive to Amoxycillin Clavulanic acid combination.This paradoxical observation warrants meticulous field studies for its approval or denial. The data is not supported by other studies which document a higher prevalence of MRSA. Continued surveillance in various departments is needed to evaluate this observation.

AMS PATTERN OF PSEUDOMONAS ISOLATES

Pseudomonas aeruginosa is the next common organism isolated from the lesions in this study constituting 17% of the total isolates. All the isolates are resistant to Ampicillin, Amoxycillin, Cotrimoxazole, Doxycycline, Cephelexin and Cefixime while all are susceptible to Piperacillin-Tazobactum and Imipenam. Prevalence of ESBL producers is 21.5%. There was a higher incidence of Pseudomonas in patients who stayed for longer periods in the hospital. Piperacillin-Tazobactum and Imipenam remain the choice of antimicrobial for the MDR isolates.

Incidence of Enterobacter among the total isolates is 15% which is comparatively higher than the earlier studies. A simple motility examination improved the number of isolates differentiating from Klebsiella species. ESBL production among the Enterobacter isolates is 42% that needs concern. All the isolates are resistant to Amikacin. Piperacillin-Tazobactum and Imipenam are the available drugs for management.

Uniform resistance of the organisms to Ampicillin, Amoxycillin and Cefixime warrants special mention in view of them being the first line drug in almost all the Primary Health Centres and over the counter availability for all undiagnosed infections.

CONCLUSION

This study presents a comprehensive clinical and bacteriological survey of diabetic foot infection in our locality. The non-availability of local data regarding the profile of organisms and their antimicrobial sensitivity pattern is a stimulus for this study.

Though earlier data suggest the Gram Positive aerobic bacteria as predominant isolates from infected diabetic foot ulcers, the aerobic Gram negative bacilli are the most frequently isolated. Hence the major etiological factors for DFI in our patients are different. Isolation of multidrug resistant Pseudomonas aeruginosa and increasing fraction of Enterobacter species raises a serious concern about the treatment modality.

Higher prevalence of both MRSA isolates and ESBL producing Gram negative organisms confirms that MDRO infection is alarmingly higher in our patients on treatment for diabetic foot infection. The place of study being a referral centre with fluent usage of broad spectrum antibiotics and the non compliance of the patients to prolonged treatment may be the possibility behind.

The increased duration of Diabetes per se, prolonged hospital stay, poor glycemic control , associated peripheral vascular disease in addition to neuropathy are identified as significant risk factors for MDRO infections in this study that is also supported by earlier Indian and global studies as mentioned earlier . The need for surgical management is found to be more in these cases. This finding suggests the necessity to develop an effective economical empirical antimicrobial policy tailored to the local data obtained and discussed here.

Trivial measures like earlier assessment of neuropathy, education about foot care &foot-wear, awareness about minor infections and foot hygiene ill have major impact in the course and management of Diabetic foot infections.

Apart from derivation of an empirical antimicrobial regimen this study emphasizes the necessity for development of DIABETIC FOOT CARE TEAM comprising of medical, surgical, paramedical and infectious disease specialists to implement the recommended multidisciplinary approach in the management of Diabetic foot infection.

INFORMED CONSENT

I have been informed about the study of Diabetic Foot Infection. I totally agree to participate in the study, as I realize the importance of the study. I am also aware that I can withdraw from the study whenever I want.

Date : Signature of the patient

Department :

STUDY ON CLINICOMICROBIOLOGICAL PROFILE OF DIABETIC FOOT INFECTIONS

PATIENT PROFORMA

Name: **Age/Sex:** **Ward/Unit:**

OP/IP No.: **CENTRAL LAB No:** **MICRO No:**

Type of Diabetes:

Duration of Diabetes:

Glycemic control:

Associated Complications:

i) Retinopathy -

ii) Neuropathy -

iii) Nephropathy –

iv) Hypertension –

v) Peripheral vascular disease:

Duration & Size of Ulcer:

Duration of Hospital stay:

Clinical Outcome:

WORKSHEET

Specimen	:	Pus, Aspirate
Method of collection	:	Deep swabbing, Aspiration, Curetting
I. Macroscopic Examination	:	Consistency, Presence of blood, Colour, Odour
II. Microscopic Examination	:	Direct Gram staining
III. Culture	:	
Nutrient agar	:	
MacConkey agar	:	
Blood agar	:	
Gram staining	:	
Motility	:	
IV. Biochemical Reactions:		
Catalase	:	
Oxidase	:	
Sugar fermentation tests	:	
IMViC	:	
Urease	:	
TSI	:	
LAO	:	Special Tests:
Coagulase	:	
Micro organism isolated	:	
V. Anti Microbial Susceptibility test	:	Antibiogram on MHA by Kirby Bauyer method

ANTIBIOGRAM

S.No	DRUG	A	B	C	Remarks
1.	Ampicillin				
2.	Amoxycillin				
3.	Amoxyclav				
4.	Oxacillin				
5.	Erythromycin				
6.	Azithromycin				
7.	Cotimoxazole				
8.	Doxycycline				
9.	Amikacin				
10.	Gentamicin				
11.	Ciprofloxacin				
12.	Ofloxacin				
13.	Cephelexin				
14.	Cefixime				
15.	Cefotaxime				
16.	Ceftrioxone				
17.	Ceftazidime				
18.	Ceftazidime+clav				
19.	Cefipime				
20.	Piperacillin+Tazo				
21.	Aztreonam				
22.	Imipenam				
23.	Vancomycin				

VI. Screening for MRSA

MHA with 4%Nacl :

VII. Conformation of MRSA :

Oxacillin Screen Agar :

VIII. Screening for ESBL

Double disc synergy test :

IX. Conformation of ESBL :

1. Double disc potentiation :

2. E Test :

X.Screening for Amp C β-lactamases:

1. Cefoxitin resistant strain :

2. Double disc antagonism test:

XI.Confirmation for Amp C β-lactamases:

Amp C disc test:

GRAM STAINING:

The gram stain was prepared as follows:

PRIMARY DYE:

Crystal violet - 10g

Ammonium oxalate - 4.25g

Absolute alcohol - 50ml

Distilled water -500ml

The methyl violet dye was dissolved in 50 ml absolute alcohol and mixed thoroughly. Then ammonium oxalate 4.25 g was dissolved in 100 ml of distilled water and this mixture was added to the violet stain and finally distilled water was added to make 500 ml. The total mixture was filtered before use.

Gram's iodine solution consists of the following

Iodine - 25g

KI - 50G

DW - 500ml

Fifty grams of KI was dissolved in 500 ml of water and then 25 grams of iodine was added to that. When iodine is dissolved, the solution was made up to 500ml with distilled water.

Counter stain used in grams stain was dilute carbol fuschin. It consists of the following:

Basic fuschin - 5g

Phenol -25g

Absolute alcohol -50 ml

The basic fuschin powder was added to alcohol at intervals until it was dissolved. Then phenol too was dissolved in distilled water. Both the solution was mixed in a separate container.

CATALASE TEST:

Done by both slide & tube methods.

Tube method:

A small amount of the culture was picked up from the nutrient agar plate with a clean, sterile glass rod and inserted into a tube of 3% hydrogen peroxide; there was no effervescence or bubble formation.

Slide method:

Pure growth of the organism from the agar was transferred to a clean slide with a sterile glass rod. Immediately 2 to 3 drops of 3% hydrogen peroxide was added to the growth, observed for the release of the bubbles.

MEDIA PREPARATION

1. **Peptone water:**

   Peptone 1 g

   Sodium chloride 0.5 g

   Distilled water 100 ml pH – 7.4

   Sterilise by autoclaving at 121°C for 15 minutes.

2. **Nutrient broth :**

   Peptone water 100ml

   Beef extract 1 g

   Ph 7.4

   Sterilise by autoclaving at 121°C for 15 minutes.

3. **Nutrient agar :**

   To the nutrient broth, add required amount of agar. Steam to dissolve agar, filter, and adjust ph to 7.4. Sterilise by autoclaving at 121°C for 15 min.

4. **Blood agar :**

   To the 100 ml of nutrient agar, in water bath at 50°C, add 5% (5ml) of Sheep blood.

5. Mac Conkey agar

Peptone	20 g
Sodium chloride	5 g
Sodium taurocholate	5 g
Lactose	10g
Neutral red	10 ml
Agar	15 g
Distilled water	1000 ml

Sterilise by autoclaving at 121°C for 15 minutes.

6. Mueller Hinton Agar:

Beef infusion	300 g/l
Casein acid hydrolysate	17.5 g
Starch	1.5 g
Agar	17 g
Distilled water	1000 ml

Sterilise by autoclaving at 121°C for 15 minutes.

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